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(57) Abstract

Growth hormone-regulatable brown adipose tissue genes and proteins have been identified. They may be used as diagnostic markers of pathologies of adipose tissue.

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GROWTH HORMONE-REGULATABLE BROWN ADIPOSE TISSUE GENES AND PROTEINS AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to the diagnosis of abnormal GH activity or general pathological activity in brown adipose tissue.

Description of the Background Art

Brown Adipose Tissue:

Brown adipose tissue (BAT) is known as a major site of heat production or thermogenesis where it normally consumes fat derived from white adipose tissues (WAT). Brown adipocytes generally reside in capillary beds and are abundant in cytochromes and proteins, particularly uncoupling protein-1 (UCP_1). A BAT depot is located in the interscapular space in rodents, but exists in the abdominal, neck, and upper back areas in human neonates. In contrast to rodents, BAT gradually disappears as children grow. Recent studies demonstrated that brown adipocytes can also be found in rat and baboon WAT during cold stress (Cousin et al. 1992 and Viguerie-Bascands et al. 1996).

Growth Hormones:

The growth hormones are vertebrate proteins with about 191 amino acid residues, the number varying from species to species. There are four cysteine residues, and two disulfide bridges. The 3D-structure of porcine GH is known; it is composed of four major antiparallel alpha-helices, at residues 7-34, 75-87, 106-127 and 152-183.

The 3D structure of the hGH:hGH receptor complex is also known. Each molecule of hGH binds two molecules of the receptor. hGH binds to two binding sites on hGH receptor. Helix 4, the loop residues 54-74, and, to a lesser extent, helix 1, mediate binding to binding site 1. Helix 3 mediates binding to binding site 2.

See generally Harvey, et al., Growth Hormone (CRC Press:1995). GH is synthesized and secreted by the

somatotrophic and somatomammotrophic cells of the lateral anterior pituitary. The control of GH production and secretion is complex, but is mainly under the influence of growth hormone releasing hormone (GHRH) and somatostatin, which stimulate and inhibit it, respectively. The shifting balance between these regulatory agents is responsible for the pulsatile nature of GH secretion, with normal human concentrations ranging from a baseline value < 1 µg/L to peaks of 25-50 µg/L. Glucocorticoids and thyroid hormones, and various carbohydrates, amino acids, fatty acids and other biomolecules, are also known to directly or indirectly regulate GH secretion.

Most GH is secreted at night, during deep sleep, but some is secreted in response to exercise and other forms of physical stress. About 500 µg/m2 body surface area are secreted by women, and 350 by men. GH secretion rates are highest in adolescents and lowest in the elderly. GH has a plasma half-life of about 20-25 min. and is cleared at a rate of 100-150 ml/m2 body surface area.

Metabolic and Clinical Effects of Growth Hormone:

Chronic elevation of growth hormone levels in humans usually results in either gigantism or acromegaly. GH, besides affecting skeletal growth, can also influence other organ systems, in particular, the liver and kidney. In the kidney, it has been associated with glomerulosclerosis and nephropathy. In the liver, it has been shown to cause an increase in liver size, as a consequence of both hyperplasia and hepatocyte hypertrophy. The hepatocellular lesions associated with high GH levels progress with age. See Quaife, et al, Endocrinol., 124: 49 (1989).

There is reason to believe that excessive GH activity in the liver is deleterious to health. Mice that express GH transgenes typically live to only about one year of age, while the normal life expectancy for mice is 2-2.5 years. A major cause of death in the GH transgenic mice has been liver disease.

Growth hormone (GH) is an essential regulator of

carbohydrate and lipid metabolism, participating in glucose uptake and usage, accelerating fat expenditure, preventing triglyceride accumulation, and facilitating lipid mobilization in adipose tissues. Growth patterns and body compositions of transgenic mice expressing GH analogs have been characterized in our laboratory (Knapp et al. 1994). One transgenic mouse line expresses a GH antagonist (GHA) and is dwarf. As these mice age, they become obese.

Chronic depression of GH levels can also impair health.

Growth Hormone Antagonists:

In view of the foregoing, it has been suggested that if a subject is suffering from excessive GH activity, it can be useful to inhibit such activity by inhibiting the production, release or action of GH, or facilitating the elimination of GH.

Among the agents useful for this purpose are those which are competitive binding antagonists of GH. It was discovered that certain mutants of GH are useful for this purpose. Kopchick, USP 5,350,836.

In order to determine whether it is appropriate to initiate or terminate use GH antagonists or other GH-inhibiting drugs, it is important to be able to monitor GH activity.

Monitoring of GH Activity:

The most straightforward marker of GH activity is the serum level of GH per se. For humans, the mean GH concentration (ug/L) in blood is

preadolescent	4.6	
early adolescent	4.8	
late adolescent	13.8	
adult	1.8	
ISS (10y old)	3.5	
GH deficient	1.4	
IDDM (boys)	9.0	
Obese (male)	0.66	(lower than controls)
Fasting	6.7	(higher than controls)
Hyperthyroid	1.9	(higher than controls)

ISS = idiopathic short stature, IDDM = insulin dependent diabetes mellitus
See Harvey (1995), supra.

While there is definitely a correlation between high levels of GH in serum, and high levels of GH activity, it must be recognized that both the total number of GH receptors, and the distribution of those receptors among the various organs, will vary from individual to individual. Hence, in determining whether an individual is suffering from excessive GH activity, and prone to develop adverse clinical sequelae, it is helpful to identify a metabolite which is produced or released in direct or indirect response to GH and, in particular, one which is substantially liver-specific so that the specific threat to liver function can be assessed.

Another marker of GH activity is insulin-like growth factor-1 (IGF-1). IGF-1 is a 70 amino acid single chain protein, with some structural similarity to proinsulin, which is closely regulated by GH secretion. While the majority of IGF-1 synthesis occurs in the liver, many other tissues, including bone and skeletal muscle, also release IGF-1 in response to GH. IGF-1 levels have been used by clinicians to confirm suspected cases of acromegaly.

However, it would be desirable to have a marker, or combination of markers, which was more liver specific than IGF-1, for use in monitoring and predicting the effect of chronic elevation of GH levels on liver function.

SUMMARY OF THE INVENTION

Applicants have identified certain genes whose expression in brown adipose tissue is elevated or depressed as a result of higher than normal GH levels.

By use of nucleic acid binding agents to bind messenger RNA transcripts produced by the transcription of any of these genes (or to bind the corresponding complementary DNAs synthesized in vitro), or by use of a protein binding agent to bind a protein encoded by any of these genes, it is possible to assay the level of transcription of the gene in question, or the level of expression and secretion of the corresponding protein, and to correlate such level with the level of GH activity in brown adipose tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Analysis of Brown Adipose Tissue Growth in Transgenic Mice. In upper panel (A), 10-week old male GHA mouse has remarkably-enlarged size of iaT ($p = 0.0131$), iBAT ($p = 3.5 \times 10^{-6}$), and iWAT ($p = 0.0155$) comparing to their nontransgenic littermates. This overgrowth has been observed at least at a similar significant level for iAT ($p = 2.0 \times 10^{-6}$), iBAT ($p = 5.8 \times 10^{-16}$), and iWAT ($p = 4.4 \times 10^{-5}$) if normalized by their body weights. In lower panel (B), since the body size of GHR/BP is about 51.8% of NT littermate, the size for those adipose tissues are not significantly different from NT littermate by gram. However, when normalized by its body weight in percentage, the enlargement has been observed at a significant level for iAT ($p = 4.5 \times 10^{-4}$), iBAT ($p = 5.8 \times 10^{-16}$), and iWAT ($p=1.0 \times 10^{-5}$). In both dwarf mice, the size of eWAT tends to be proportional to that of entire body by weight. The significant difference for eWAT weight ($p = 2.8 \times 10^{-5}$ in GHA group and $p = 0.0013$ in GHR/BPKO group) may be due to their small body size. Although iBAT weight of bGH mouse is greater than that of NT littermate ($p = 0.0250$), normalized iBAT weight in percentage does not exhibit any significant difference for iAT, iBAT, iWAT, and eWAT, suggesting that, in bGH mice, those adipose tissues grow rather proportionally to the body weight and that any impairment of GH signaling may result in an overlarged size of interscapular adipose tissue which constitutes of iBAT and iWAT.

Figure 2 Northern Blot of UCP1 in BAT from Different Transgenic Mouse. Hybridizing total RNA prepared from various tissues of 10-week old male and female NT mice with 605-bp probe, a portion of UCP1 ORF sequence, the UCP1 signals are only observed in iBAT in male transgenic mice and NT littermates after 2-hr exposure even prolonged exposure. The mRNA level of UCP1 is enhanced in GHA and GHR/BPKO mice and is reduced in bGH mice when comparing that in NT littermates. The ratio of intensity volume of UCP1 to β-actin demonstrates that these changes are substantial.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

We have found that the BAT mass in the GHA mice (n=17) is significantly greater (p =0.011) than that found in their non-transgenic (NT) littermates (n=24) when normalized for body weight. Hence, we proposed that genes in BAT may be up or down-regulated by GH.

To examine this hypothesis, we employed a PCR-select cDNA subtraction assay (Clontech) and constructed a forward subtraction library by subtracting NT littermate BAT cDNAs from GHA BAT cDNAs and a reverse subtraction library by subtracting GHA BAT cDNAs from NT BAT cDNAs. Positive clones were screened by differential hybridization using probes made from the two subtracted cDNA libraries. Partial cDNAs were isolated, sequenced, and analyzed by BLAST searches. We found genes encoding glucosephosphate isomerase, α-enolase, pyruvate kinase, proteasome, ubiquitin, and heme oxygenase in the forward subtraction library, indicating that these genes are up-regulated in GHA BAT. We found genes encoding mitochondria cytochrome b, mitochondria cytochrome C oxidase subunit I, mitochondria NADH-ubiquinone oxidoreductase chain 4 and/or 6, medium chain acyl-CoA dehydrogenase, adipocyte lipid binding protein, and trans-Golgi network in the reverse subtraction library, indicating that these genes are down-regulated in GHA BAT. These results may partially explain why GHA mice become obese.

The present invention relates to the use of these genes and proteins as diagnostic markers in the analysis of brown adipose tissue structure and function, in particular, its differentiation, theriogenesis and pathologies.

It is now possible to determine the level of the mRNAs or proteins corresponding to these genes, in normal adipose tissue as compared to adipose tissue in a pathological state, and thereby determine reference values of these mRNAs or proteins which are indicative of a particular pathological state.

Known pathologic lesions in adipose tissues include:

white adipose tissues:

Aging; insulin resistance; hyperlipidemia; non-insulin dependent diabetes mellitus; obesity;

benign and malignant tumor

Brown adipose tissue and brown adipocyte;
Aging; insulin resistance; hyperlipidemia; non-insulin dependent diabetes mellitus; obesity; benign and malignant tumor

Convertible adipose tissues and convertible adipocytes:
Aging; insulin resistance; hyperlipidermia; non-insulin dependent diabetes mellitus; obesity; benign and malignant tumor.

The preferred screening assay for this purpose is an antisense probe assay.

It additionally may be of advantage to ascertain the level of the mRNAs and proteins in cells of the liver, kidney, muscle, heart, spleen, intestine, brain, lung, testis, and ovary, and correlate the level with a particular pathological condition.

Definitions

Two proteins are cognate if they are produced in different species, but are sufficiently similar in structure and biological activity to be considered the equivalent proteins for those species. If the accepted scientific names for two proteins are the same but for the species identification (e.g., human GH and shark GH), they should be considered cognate. If not, the two proteins may still be considered cognate if they have at least 50% amino acid sequence identity (when globally aligned with a pam250 scoring matrix with a gap penalty of the form q+r(k-1) where k is the length of the gap, q=-12 and r=-4; percent identity=number of identities as percentage of length of shorter sequence) and at least one biological activity in common.

Two genes are cognate if they are expressed in different species and encode cognate proteins.

Gene expression may be said to be specific to a particular tissue if the average ratio of the specific mRNA to total mRNA for the cells of that tissue is at least 10% higher than the average ratio is for the cells of some second tissue. Absolute specificity is not required. Hence, a gene may be said to be

expressed specifically in more than one tissue.

When the term "specific" is used in this specification, absolute specificity is not intended, merely a detectable difference.

Preferably the markers of the present invention are, singly or in combination, more specific to the target tissue than are serum GH or IGF-1 levels, or than GH mRNA or IGF-1 mRNA levels in the target tissue.

If this specifications calls for alignment of DNA sequences, and one of the sequences is intended for the use as a hybridization probe, the sequences are to be aligned using a local alignment program with matches scored +5, mismatches scored -4, the first null of a gap scored -12, and each additional null of the same gap scored -2. Percentage identity is the number of identities expressed as a percentage of the length of the overlap, including internal gaps.

In Vitro Assays

The in vitro assays of the present invention may be applied to any suitable analyte-containing sample, and may be qualitative or quantitative in nature.

For the techniques to practice these assays, see, in general, Ausubel, et al., Current Protocols in Molecular Biology, and in particular chapters 2 ("Preparation and Analysis of DNA"), 3 ("Enzymatic Manipulation of DNA and RNA"), 4 ("Preparation and Analysis of RNA"), 5 ("Construction of Recombinant DNA libraries") 6 ("Screening of Recombinant DNA Libraries"), 7 ("DNA Sequencing"), 10 ("Analysis of Proteins"), 11 ("Immunology"), 14 ("In situ hybridization and immune histochemistry"), 15 ("The Polymerase Chain Reaction"), 19 ("Informatics for Molecular Biologists"), and 20 ("Analysis of Protein Interactions"). Also see, in general, Coligan, et al., Current Protocols in Immunology, and in particular, chapters 2 ("Induction of immune responses"), 8 ("Isolation and Analysis of Proteins"), 9 ("Peptides"), 10 ("Molecular Biology") and 17 ("Engineering Immune Molecules and Receptors"). Also see Coligan, et al., Current Protocols in Protein Science.

The Assay Target (Analyte)

The assay target may be a positive or negative marker. A positive marker is one for which a higher signal is correlated with abnormally high growth hormone activity. A negative marker is one for which a higher signal is correlated with abnormally low growth hormone activity. Positive markers are up-regulated in high GH mammals and down-regulated in low GH mammals. Negative markers are up-regulated in high GH mammals and down-regulated in low GH mammals.

A mammal which expresses a GH antagonist (GHA) is normally considered a low GH level, because it expresses the endogenous GH at presumably normal levels but the overall GH activity is depressed as a result of the co-expression of the GHA.

Hence, genes which are up-regulated in GHA mice are actually negative markers, while genes which are down-regulated in GHA mice are actually positive markers.

In one embodiment, the assay target is a messenger RNA transcribed from a gene which, in brown adipose tissue, has increased transcriptional activity if serum GH levels are increased. This messenger RNA may be a full length transcript of the gene, or merely a partial transcript. In the latter case, it must be sufficiently long so that it is possible to achieve specific binding, e.g., by nucleic acid hybridization. For the purpose of conducting the assay, the messenger RNA is extracted from brown adipose tissue by conventional means. Alternatively, the assay target may be a complementary DNA synthesized in vitro from the messenger RNA as previously described.

For convenience, the term "gene" or "target sequence" will be used to refer to both the messenger RNA or complementary DNA corresponding to the induced gene, and to the coding gene proper.

In another embodiment, the assay target is a protein encoded by said gene and expressed at higher levels in response to elevated GH levels. If the protein is secreted, the assay may be performed on serum. If the protein is not secreted, then cells of brown adipose tissue will be obtained from the subject and lysed to expose the cytoplasmic contents.

In either embodiment, one or more purification steps may be employed prior to the practice of the assay in order to enrich the sample for the assay target.

The proteins of particular interest are as follows:

Negative Markers:
glucosephosphate isomerase
neuroleukin
pyruvate kinase
heme oxygenase
ubiquitin/ribosomal fusion protein
α-enolase
proteasome θ chain

Positive Markers
trans-Golgi network protein
medium chain acyl-CoA dehydrogenase
adipocyte lipid binding protein
cytochrome c oxidase
NADH-ubiquonone oxidoreductase
cytochrome b

The genes of particular interest are those encoding the above proteins. These genes were identified, as described in Example 1, on the basis of the identity or similarity of mouse cDNAs obtained by subtractive hybridization methods to known mouse genes or cDNAs. The mouse sequences are set forth in the figures. However, if the assay is of a human subject, the target gene or protein will of course be the cognate human gene or protein. The sequence databank ID numbers for these cognate human genes and proteins are given in Table A.

Certainly newly discovered DNAs are also of interest as positive markers. These are identified below as clones

Ng-G119K2
Ng-G119K15
Ng-G119K36
Ng-G119K62
Ng-G119K42

Ng-G119K58
Ng-G119K65
Ng-G119K66

The proteins encoded by the ORFs embedded in these DNAs are also of interest.

Samples

The sample may be of any biological fluid or tissue which is reasonably expected to contain the messenger RNA transcribed from one of the above genes, or a protein expressed from one of the above genes. The sample may be of brown adipose tissue or interstitial fluid, or of a systemic fluid into which brown adipose tissue proteins are secreted.

A non-invasive sample collection will involve the use of urine samples from human subjects. Blood samples will also be obtained in order to obtained plasma or serum from which secreted proteins can be evaluated. Brown adipose tissue aspirates can also be obtained to detect for the presence of genes and proteins of interest. The most invasive method would involve obtaining brown adipose tissue biopsies.

Analyte Binding Reagents (Molecules, ABM)

When the assay target is a nucleic acid, the preferred binding reagent is a complementary nucleic acid. However, the nucleic acid binding agent may also be a peptide or protein. A peptide phage library may be screened for peptides which bind the nucleic acid assay target. In a similar manner, a DNA binding protein may be randomly mutagenized in the region of its DNA recognition site, and the mutants screened for the ability to specifically bind the target. Or the hypervariable regions of antibodies may be mutagenized and the antibody mutants displayed on phage.

When the assay target is a protein, the preferred binding reagent is an antibody, or a specifically binding fragment of an antibody. The antibody may be monoclonal or polyclonal. It can be obtained by first immunizing a mammal with the protein target, and recovering either polyclonal antiserum, or immunocytes for later fusion to obtain hybridomas, or by

constructing an antibody phage library and screening the antibodies for binding to the target. The binding reagent may also be a binding molecule other than an antibody, such as a receptor fragment, an oligopeptide, or a nucleic acid. A suitable oligopeptide or nucleic acid may be identified by screening a suitable random library.

Binding and Reaction Assays

The assay may be a binding assay, in which one step involves the binding of a diagnostic reagent to the analyte, or a reaction assay, which involves the reaction of a reagent with the analyte. The reagents used in a binding assay may be classified as to the nature of their interaction with analyte: (1) analyte analogues, or (2) analyte binding molecules (ABM). They may be labeled or insolubilized.

In a reaction assay, the assay may look for a direct reaction between the analyte and a reagent which is reactive with the analyte, or if the analyte is an enzyme or enzyme inhibitor, for a reaction catalyzed or inhibited by the analyte. The reagent may be a reactant, a catalyst, or an inhibitor for the reaction.

An assay may involve a cascade of steps in which the product of one step acts as the target for the next step. These steps may be binding steps, reaction steps, or a combination thereof.

Signal Producing System (SPS)

In order to detect the presence, or measure the amount, of an analyte, the assay must provide for a signal producing system (SPS) in which there is a detectable difference in the signal produced, depending on whether the analyte is present or absent (or, in a quantitative assay, on the amount of the analyte). The detectable signal may be one which is visually detectable, or one detectable only with instruments. Possible signals include production of colored or luminescent products, alteration of the characteristics (including amplitude or polarization) of absorption or emission of radiation by an assay component or product, and precipitation or agglutination

of a component or product. The term "signal" is intended to include the discontinuance of an existing signal, or a change in the rate of change of an observable parameter, rather than a change in its absolute value. The signal may be monitored manually or automatically.

In a reaction assay, the signal is often a product of the reaction. In a binding assay, it is normally provided by a label borne by a labeled reagent.

Labels

The component of the signal producing system which is most intimately associated with the diagnostic reagent is called the "label". A label may be, e.g., a radioisotope, a fluorophore, an enzyme, a co-enzyme, an enzyme substrate, an electron-dense compound, an agglutinable particle.

The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are ^{3}H, ^{32}P, ^{125}I, ^{131}I, ^{35}S, ^{14}C, and, preferably, ^{125}I.

The label may also be a fluorophore. When the fluorescently labeled reagent is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, fluorescence-emitting metals such as ^{125}Eu, or others of the lanthanide series, may be incorporated into a diagnostic reagent using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) of ethylenediamine-tetraacetic acid (EDTA).

The label may also be a chemiluminescent compound. The presence of the chemiluminescently labeled reagent is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isolumino, theromatic acridinium ester, imidazole,

acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used for labeling. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Enzyme labels, such as horseradish peroxidase and alkaline phosphatase, are preferred. When an enzyme label is used, the signal producing system must also include a substrate for the enzyme. If the enzymatic reaction product is not itself detectable, the SPS will include one or more additional reactants so that a detectable product appears.

An enzyme analyte may act as its own label if an enzyme inhibitor is used as a diagnostic reagent.

Conjugation Methods

A label may be conjugated, directly or indirectly (e.g., through a labeled anti-ABM antibody), covalently (e.g., with SPDP) or noncovalently, to the ABM, to produce a diagnostic reagent.

Similarly, the ABM may be conjugated to a solid phase support to form a solid phase ("capture") diagnostic reagent.

Suitable supports include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention.

The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to its target. Thus the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc.

Binding Assay Formats

Binding assays may be divided into two basic types, heterogeneous and homogeneous. In heterogeneous assays, the interaction between the affinity molecule and the analyte does not affect the label, hence, to determine the amount or presence of analyte, bound label must be separated from free label. In homogeneous assays, the interaction does affect the activity of the label, and therefore analyte levels can be deduced without the need for a separation step.

In one embodiment, the ABM is insolubilized by coupling it to a macromolecular support, and analyte in the sample is allowed to compete with a known quantity of a labeled or specifically labelable analyte analogue. The "analyte analogue" is a molecule capable of competing with analyte for binding to the ABM, and the term is intended to include analyte itself. It may be labeled already, or it may be labeled subsequently by specifically binding the label to a moiety differentiating the analyte analogue from analyte. The solid and liquid phases are separated, and the labeled analyte analogue in one phase is quantified. The higher the level of analyte analogue in the solid phase, i.e., sticking to the ABM, the lower the level of analyte in the sample.

In a "sandwich assay", both an insolubilized ABM, and a labeled ABM are employed. The analyte is captured by the insolubilized ABM and is tagged by the labeled ABM, forming a ternary complex. The reagents may be added to the sample in either order, or simultaneously. The ABMs may be the same or different. The amount of labeled ABM in the ternary complex is directly proportional to the amount of analyte in the sample.

The two embodiments described above are both heterogeneous assays. However, homogeneous assays are conceivable. The key is that the label be affected by whether or not the complex is formed.

Detection of Genes of Interest

For the detection of genes in the sample, **PCR** can be done using primers specific for the genes of interest. This would

amplify the genes of interest. Primers may be designed to anneal to any site within the open reading frames of the genes of interest. Resolution of the fragments by electrophoresis on agarose gel may be used to determine the presence of the genes. **PCR** product may be quantitated by densitometry in order to estimate the concentration of the genes in the samples.

Detection of genes of interest may also be done by **Northern blot analysis** on liver *biopsies*. Tissue sample from patients may be obtained and the total RNA extracted using RNAStat 60. The total RNA sample may then be resolved on denaturing gel by electrophoresis and then transferred onto a nylon membrane. After transfer of RNA onto the membrane, the membrane may then be used in hybridization with a suitable probe, which may be a synthetic probe directed against a gene already known to be a marker, or which may be a cDNA probe prepared directly from subtractive hybridization, wherein the fragment encoding the gene of interest, that is enriched in GH-overproducing subjects, will be labeled, preferably either radioactively with ^{32}P or non-radioactively with DIG (Digoxigenin). A negative control, such as one composed of RNA sample from brown adipose tissue of normal subjects, may be resolved side by side with the patients' sample, to determine quantitatively whether there is a significant increase in the level of gene expression. Elevation of the messenger RNA transcript from this gene would imply that brown adipose tissue damage might have occurred.

The DNA sequences of the present invention may be used either as hybridization probes per se, or as primers for PCR.

In a hybridization assay, a nucleic acid reagent may be used either as a probe, or as a primer. For probe use, only one reagent is needed, and it may hybridize to all or just a part of the target nucleic acid. Optionally, more than one probe may be used to increase specificity. For the primer-based assay, two primers are needed. These hybridize the non-overlapping, separated segments of the target sequence. One primer hybridizes to the plus strand, and the other to the minus strand. By PCR techniques, the target nucleic acid region starting at one primer binding site and ending at the

other primer binding site, along both strands, is amplified, including the intervening segment to which the primers do not hybridize. In a primer-based assay, the primer thus will not correspond to the entire target, but rather each primer will correspond to one end of the target sequence.

In probe-based assays, hybridizations may be carried out on filters or in solutions. Typical filters are nitrocellulose, nylon, and chemically-activated papers. The probe may be double stranded or single stranded, however, the double stranded nucleic acid will be denatured for binding.

To be successful, a hybridization assay, whether primer- or probe-based, must be sufficiently sensitive and specific to be diagnostically useful.

For probe-based assays, sensitivity is affected by the amount and specific activity of the probe, the amount of the target nucleic acid, the detectability of the label, the rate of hybridization, and the duration of the hybridization. The hybridization rate is maximized at a Ti (incubation temperature) of 20-25°C. below Tm for DNA:DNA hybrids and 10-15°C. below Tm for DNA:RNA hybrids. It is also maximized by an ionic strength of about 1.5M Na^+. The rate is directly proportional to duplex length and inversely proportional to the degree of mismatching.

For primer-based PCR assays, sensitivity is not usually a major issue because of the extreme amplification of the signal.

For probe-based assays, specificity is a function of the difference in stability between the desired hybrid and "background" hybrids. Hybrid stability is a function of duplex length, base composition, ionic strength, mismatching, and destabilizing agents (if any).

The Tm of a perfect hybrid may be estimated.

for DNA:DNA hybrids, as

$$Tm = 81.5°C + 16.6\ (\log M) + 0.41\ (\%GC) - 0.61\ (\%\ form) - 500/L$$

and for DNA:RNA hybrids, as

$$Tm = 79.8°C + 18.5\ (\log M) + 0.58\ (\%GC) - 11.8\ (\%GC)^2 - 0.56(\%\ form) - 820/L$$

where

M, molarity of monovalent cations, 0.01-0.4 M NaCl,

%GC, percentage of G and C nucleotides in DNA, 30%-75%,

% form, percentage formamide in hybridization solution, and

L, length hybrid in base pairs.

Tm is reduced by 0.5-1.5°C for each 1% mismatching.

Tm may also be estimated by the method of Tinoco et al., developed originally for the determination of the stability of a proposed secondary structure of an RNA. Tm may also be determined experimentally.

Filter hybridization is typically carried out at 68°C., and at high ionic strength (e.g., 5 - 6 x SSC), which is nonstringent, and followed by one or more washes of increasing stringency, the last was being of the ultimately desired stringency. The equations for Tm can be used to estimate the appropriate Ti for the final wash, or the Tm of the perfect duplex can be determined experimentally and Ti then adjusted accordingly.

While a mouse cDNA was used to probe a mouse liver cDNA library, and could be used to probe nonmurine liver cDNA libraries, it would be expected that there would be some sequence divergence between cognate mouse and nonmouse DNAs, possibly as much as 25-50%.

Hence, when the human DNA cognate to the original mouse cDNA is known, it is better to use that DNA, or a fragment thereof, to probe a human liver cDNA library. The practitioner may use the complete genomic DNA or cDNA sequence of the human gene as a probe, or, for the sake of greater specificity or synthetic convenience, a partial sequence.

It is also noted that while some of the mouse clones were identical to subsequences of a databank mouse DNA, others diverged slightly (up to 5%). This divergence could be artifactual (sequencing error) or real (allelic variation).

Hybridization conditions should be chosen so as to permit allelic variations, but avoid hybridizing to other genes. In general, stringent conditions are considered to be a Ti of 5°C.

below the Tm of a perfect duplex, and a 1% divergence corresponds to a 0.5-1.5°C. reduction in Tm. Typically, the mouse clones were 95-100% identical to database mouse sequences. Hence, use of a Ti of 5-15°C. below, more preferably 5-10°C. below, the Tm of the double stranded form of the probe is recommended.

If the sequences of the major allelic variants are known, one may use a mixed probe, and optionally increase the stringency.

If there is no known human gene cognate to the mouse (or rat) gene homologous to the clone, then the mouse (or rat) gene, or other known nonhuman cognate gene, may be used as a probe. In this case, more moderate stringency hybridization conditions should be used. The nonhuman gene may be modified to obey a more human set of codon preferences.

Alternatively, the mouse (or rat) gene may be used once as a probe to isolate the human gene, and the human gene then used for diagnostic work. If a partial human cDNA is obtained, it may be used to isolate a larger human cDNA, and the process repeated as needed until the complete human cDNA is obtained.

For cross-species hybridization, the Ti should be reduced further, by about 0.5-1.5°C, e.g., 1°C, for each expected 1% divergence in sequence. The degree of divergence may be estimated from the known divergence of the most closely related pairs of known genes from the two species.

If the desired degree of mismatching results in a wash temperature less than 45°C., it is desirable to increase the salt concentration so a higher temperature can be used. Doubling the SSC concentration results in about a 17°C. increase in Tm, so washes at 45°C in 0.1 x SSC and 62°C in 0.2 x SSC are equivalent (1 x SSC = 0.15 M NaCl, 0.015M trisodium citrate, pH 7.0).

The person skilled in the art can readily determine suitable combinations of temperature and salt concentration to achieve these degrees of stringency.

The hybridization conditions set forth in the examples may be used as a starting point, and then made more or less stringent as the situation merits.

Examples of successful cross-species-hybridization experiments include Braun, et al., EMBO J., 8:701-9 (1989) (mouse v. human), Imamura, et al., Biochemistry, 30:5406-11 (1991) (human v. rat), Oro, et al, Nature, 336:493-6 (1988) (human v. Drosophila), Higuti, et al., Biochem. Biophys. Res. Comm., 178:1014-20 (1991) (rat v. human), Jeung, et al., FEBS Lett., 307:224-8 (1992) (rat, bovine v. human), Iwata, et al., Biochem. Biophys. Res. Comm., 182:348-54 (1992) (human v. mouse), Libert, et al., Biochem. Biophys. Res. Comm., 187:919-926 (1992) (dog v. human), Wang, et al., Mamm. Genome, 4:382-7 (1993) (human v. mouse), Jakubiczka, et al., Genomics, 17:732-5 (1993) (human v. bovine), Nahmias, et al., EMBO J., 10:3721-7 (1991) (human v. mouse), Potier, et al., J. DNA Sequencing and Mapping, 2:211-218 (1992) (rat v. human), Chan, et al., Somatic Cell Molec. Genet., 15:555-62 (1989) (human v. mouse), Hsieh, et al., Id., 579-590 (1989) (human, mouse v. bovine), Sumimoto, et al., Biochem. Biophys. Res. Comm., 165:902-6 (1989) (human v. mouse), Boutin, et al., Molec. Endocrinol., 3:1455-61 (1989) (rat v. human), He, et al., Biochem. Biophys. Res. Comm., 171:697-704 (1990) (human, rat v. dog, guinea pig, frog, mouse), Galizzi, et al., Int. Immunol., 2:669-675 (1990) (mouse v. human). See also Gould, et al., Proc. Nat. Acad. Sci. USA, 86:1934-8 (1989).

In general, for cross-species hybridization, Ti = 25-35°C. below Tm. Wash temperatures and ionic strengths may be adjusted empirically until background is low enough.

For primer-based PCR assays, the specificity is most dependent on reagent purity.

The final considerations are the length and binding site of the probe. In general, for probe-based assays, the probe is preferably at least 15, more preferably at least 20, still more preferably at least 50, and most preferably at least 100 bases (or base pairs) long. Preferably, if the probe is not complementary to the entire gene, it targets a region low in allelic variation.

In general, for primer-based PCR assays, the primer is preferably at least 18-30 bases in length. Longer primers do no harm, shorter primers may sacrifice specificity. The

distance between the primers may be as long as 10 kb, but is preferably less than 3kb, and of course should taken into account the length of the target sequence (which is likely to be shorter for mRNA or cDNA than for genomic DNA). Preferably, primers have similar GC content, minimal secondary structure, and low complementarily to each other, particularly in the 3' region. Also, their targets are preferably relatively invariant from allete to allete.

For theoretical analysis of probe design considerations, see Lathe, et al., J. Mol. Biol., 183:1-12 (1985).

Detection of Proteins of Interest

ELISA can be done on blood plasma or serum from patients using antibodies specific to the protein of interest. Samples will be incubated with primary antibodies on plates. This primary antibody is specific to the protein of interest.

Another method that can be conducted will involve the use of chemical or enzymatic reactions in which the protein of interest will act as a substrate (or, if the protein is an enzyme, as a catalyst) to cause a reaction that lead to the production of colored solution or emission of fluorescence. **Spectrometric** analysis can be done in order to determine the concentration of the proteins in the sample.

Western blot analysis can also be done on the *plasma/serum, tissue aspirate, tissue biopsies or urine samples*. This would involve resolving the proteins on an electrophoretic gel, such as an SDS PAGE gel, and transferring the resolved proteins onto a nitrocellulose or other suitable membrane. The proteins are incubated with a target binding molecule, such as an antibody.

This binding reagent may be labeled or not. If it is unlabeled, then one would also employ a secondary, labeled molecule which binds to the binding reagent. One approach involves avidinating one molecule and biotinylating the other. Another is for the secondary molecule to be a secondary antibody which binds the original binding reagent.

To improve detection of the specific protein, **immunoprecipitation** can be conducted. This typically will

involve addition of a monoclonal antibody against the protein of interest to samples, then allowing the Ig-protein complex to precipitate after the addition of an affinity bead (ie antihuman Ig sepharose bead). The immunoprecipitates will undergo several washings prior to transfer onto a nitrocellulose membrane. The Western blot analysis can be perform using another antibody against the primary antibody used.

Interpretation of Assay Results

The assay may be used to predict the clinical state of the brown adipose tissue if the level of GH activity remains unchanged.

A scheme for the diagnostic interpretation of the level of the target in question is determined in a conventional manner by monitoring the level of GH, the level of the target, and the brown adipose tissue condition in a suitable number of patients, and correlating the level of the target at an earlier time point with the simultaneous or subsequent brown adipose tissue state.

This correlation is then used to predict the future clinical state of the brown adipose tissue in new patients with high GH levels.

The diagnosis may be based on a single marker, or upon a combination of markers, which may include, besides the markers mentioned above, the level of GH or of IGF-1. A suitable combination may be identified by any suitable technique, such as multiple regression, factor analysis, or a neural network using the scaled levels of the markers as inputs and the current or subsequent brown adipose tissue state as an output.

In vivo Diagnostic Uses

Radio-labelled ABM which are not rapidly degraded in blood may be administered to the human or animal subject. Administration is typically by injection, e.g., intravenous or arterial or other means of administration in a quantity sufficient to permit subsequent dynamic and/or static imaging using suitable radio-detecting devices. The dosage is the

smallest amount capable of providing a diagnostically effective image, and may be determined by means conventional in the art, using known radio-imaging agents as a guide.

Typically, the imaging is carried out on the whole body of the subject, or on that portion of the body or organ relevant to the condition or disease under study. The amount of radio-labelled ABM accumulated at a given point in time in relevant target organs can then be quantified.

A particularly suitable radio-detecting device is a scintillation camera, such as a gamma camera. A scintillation camera is a stationary device that can be used to image distribution of radio-labelled ABM. The detection device in the camera senses the radioactive decay, the distribution of which can be recorded. Data produced by the imaging system can be digitized. The digitized information can be analyzed over time discontinuously or continuously. The digitized data can be processed to produce images, called frames, of the pattern of uptake of the radio-labelled ABM in the target organ at a discrete point in time. In most continuous (dynamic) studies, quantitative data is obtained by observing changes in distributions of radioactive decay in target organs over time. In other words, a time-activity analysis of the data will illustrate uptake through clearance of the radio-labelled binding protein by the target organs with time.

Various factors should be taken into consideration in selecting an appropriate radioisotope. The radioisotope must be selected with a view to obtaining good quality resolution upon imaging, should be safe for diagnostic use in humans and animals, and should preferably have a short physical half-life so as to decrease the amount of radiation received by the body. The radioisotope used should preferably be pharmacologically inert, and, in the quantities administered, should not have any substantial physiological effect.

The ABM may be radio-labelled with different isotopes of iodine, for example ^{123}I, ^{125}I, or ^{131}I (see for example, U.S. Patent 4,609,725). The extent of radio-labeling must, however be monitored, since it will affect the calculations made based on the imaging results (i.e. a diiodinated ABM will result in

twice the radiation count of a similar monoiodinated ABM over the same time frame).

In applications to human subjects, it may be desirable to use radioisotopes other than ^{125}I for labelling in order to decrease the total dosimetry exposure of the human body and to optimize the detectability of the labelled molecule (though this radioisotope can be used if circumstances require). Ready availability for clinical use is also a factor. Accordingly, for human applications, preferred radio-labels are for example, ^{99m}Tc, ^{67}Ga, ^{68}Ga, ^{90}Y, ^{111}In, ^{113m}In, ^{123}I, ^{186}Re, ^{188}Re or ^{211}At.

The radio-labelled ABM may be prepared by various methods. These include radio-halogenation by the chloramine - T method or the lactoperoxidase method and subsequent purification by HPLC (high pressure liquid chromatography), for example as described by J. Gutkowska et al in "Endocrinology and Metabolism Clinics of America: (1987) 16 (1):183. Other known method of radio-labelling can be used, such as IODOBEADS™.

There are a number of different methods of delivering the radio-labelled ABM to the end-user. It may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. Because proteins are subject to bering digested when administered orally, parenteral administration, i.e., intravenous subcutaneous, intramuscular, would ordinarily be used to optimize absorption of an ABM, such as an antibody, which is a protein.

Other Uses

The markers in question may also be used to determine if the subject is suffering from or prone to develop a disorder associated with **insufficient** GH activity in the brown adipose tissue.

Presumably, in that event the positive markers will be at abnormally low levels, and the negative markers are abnormally low levels.

Examples

BLASTN searches were performed with the default parameters match +1, mismatch -3, gap q=-5 r=-2, penalty q+rk for gap length k. For BLASTP, BLOSUM62 matrix with q=-1, r=-1, lambda ratio=0.85.

Example 1

Brown Adipose Tissue (BAT) Total RNA Preparation

70-day old male G119K growth hormone antagonist (GHA) mice and their non-transgenic (NT) littermates were sacrificed by neck dislocation. Their interscapular brown adipose tissues (BAT) were immediately dissected, weighed, placed in 10 volumes of cold RNA STAT-60™ solution (TEL-TEST "B", Friendswood, Texas), and carefully homogenized on ice. Total RNAs were prepared by following the manufacture's protocol. Total RNA pellets were usually stored in 75% ethanol at ~80°C for not more than 6 months.

SMART™ PCR cDNA Synthesis

Fresh BAT total RNAs from GHA and NT mice were prepared with RNA STAT-60™ kit (TEL-TEST "B", Friendswood, Texas) and further purified with QIAEX© II Rneasy Mini Kit (Qiagen, Chatsworth, California). Purified total RNAs were quantified by their spectrum ratio of A260/A280 and their band intensity ratio of 18S/28S on 1% formaldehyde-Agarose gel. 1 µg of each purified total RNA (as starting material) was then applied to first-strand cDNA synthesis with SMART™ PCR cDNA Synthesis Kit (CLONTECH, Palo Alto, California). Major components, cDNA synthesis (CDS) primer ($AAGCAGTGGTAACAACGCAGAGTACT_{(30)}N_{-1}N$), SMART II oligonucleotide (AAGCAGTGGTAACAACGCAGAGTACGCGGG), and MMLV reverse transcriptase (Gibco BRL, Palo Alto, California), were included in these reactions. The second-strand cDNAs were synthesized in the presence of Advantage KlenTaq Polymerase Mix and PCR primer (AAGCAGTGGTAACAACGCAGAGT). The double-stranded (ds) cDNAs were then simultaneously amplified under the Kit-recommended PCR program (95°C for 1 minute and 15~21 cycles of 95°C for 15 seconds, 65°C for 30 seconds, and 68°C for 6

minutes). 20 for GHA and 18 for NT were determined as optimal number of PCR cycles by electrophoresing 5 µl of each PCR product on a 1.2% Agarose/EtBr gel so that all BAT SMART™ PCR cDNAs were equally synthesized for following subtraction.

PCR-Select™ cDNA Subtraction

BAT SMART™ PCR cDNAs were sized with Column chromatography and cleaved with *Rsa* I by following the protocol provided with PCR-Select™ cDNA Subtraction Kit (CLONTECH, Palo Alto, California). The digested cDNAs were purified with QIAEX II Agarose Gel Extraction Kit (Qiagen, Chatsworth, California), microfiltrated and precipitated with this Subtraction Kit. Final concentrations of 300 ng/µl were made to both GHA and NT mouse *Rsa* I-restricted BAT SMART™ PCR cDNAs.

Standard adapter ligations were performed in the presence of either adaptor 1 (CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT) or adaptor 2R (CTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGT) under the direction of PCR-Select™ cDNA Subtraction Kit so that GHA-adaptor 1, GHA-adaptor 2R, NT-adaptor 1, and NT-adaptor 2R were respectively prepared to serve as experimental tester cDNAs while unligated *Rsa* I-restricted cDNAs were used as experimental driver cDNAs.

The GHA-adaptor 1 and GHA-adaptor 2R experimental tester cDNAs were first cross-hybridized with *Rsa* I-restricted NT experimental driver cDNAs respectively; while the NT-adaptor 1 and NT-adaptor 2R tester cDNAs were done with *Rsa* I-restricted GHA driver cDNAs respectively. After 8-hour air-incubation at 68°C, the second hybridization was followed by simply mixing the first cross-hybridization products and incubating at 68°C overnight: GHA-adaptor 1 tester/NT driver and GHA-adaptor 2R/NT driver, or NT-adaptor 1 tester/GHA driver and NT-adaptor 2R/GHA driver.

The final hybridized cDNAs were primarily amplified in the presence of PCR primer 1 (CTAATACGACTCACTATAGGGC) and Advantage KlenTaq Polymerase Mix under the Kit-recommended PCR program (94°C for 25 seconds and 27-32 cycles of 94°C for 10 seconds, 66°C for 30 seconds, and 72°C for 1.5 minutes); then those PCR

cDNA products were secondarily amplified again in the presence of Nested PCR primer 1 (TCGAGCGGCCGCCCGGGCAGGT) and Nested PCR primer 2R (AGCGTGGTCGCGGCCGAGGT) for additional 9-15 cycles (94°C for 10 seconds, 68°C for 30 seconds, and 72°C for 1.5 minutes) followed by 72°C for 5 minutes. The number of primary PCR cycles were 27 for GHA and 29 for NT; while that of secondary PCR cycles were 9 for both GHA and NT. These optimal number of PCR cycles were determined by electrophoresing 8 µl of each PCR product on a 2% Agarose/EtBr gel to minimize the non-specific BAT SMART™ PCR-Select subtracted cDNA products.

Analyses of double-stranded cDNA synthesis products, *Rsa* I digestion, adaptor ligation, and subtraction efficiency were performed according to the recommendation of PCR-Select™ cDNA Subtraction Kit. End products from each manipulation were visualized on 1.2-2% Agarose/EtBr gel before proceeding to do the next step.

Subtraction Library Construction

Fresh secondary PCR amplification products after PCR-Select cDNA subtraction were ligated to 3.9 kb PCR™ II vector with a standard method provided by TA Cloning Kit (Invitrogen, Carlsbad, California). After 16-hour incubation at 14°C, these products were used to transform library efficiency DH5α™ competent cells (Life Technologies, Palo Alto, California) onto LB-ampicillin plates by using a recommended small-scale protocol. α-complementation of the β-galactosidase gene within this vector was employed to produce blue/white screening of colonies on bacterial plates containing X-gal. 160 white colonies were isolated from each subtraction library: forward subtraction library (GHA subtracting NT) and reverse subtraction library (NT subtracting GHA); total 320 colonies were maintained.

PCR-Select Differential Screening

The adaptor sequences of the secondary PCR amplification products after PCR-Select cDNA subtraction were removed by restricting with *Rsa* I digestion; and digested products were purified with QIAEX II Agarose Gel Extraction Kit (Qiagen,

Chatsworth, California) and precipitated with NH_4AC and ethanol at -20°C overnight. The down-stream products were then used to random-prime PCR Dig-labeled probes by incubating at 37°C for 5 hours with DIG High Prime DNA Labeling Kit (Boehringer Mamheim, Indianapolis, Indiana). The concentration of both forward and reverse subtraction library probes were estimated with the series dilution of Dig-labeled marker.

cDNA arrays were at mean time made with the PCR-Select Differential Screening Kit (CLONTECH, Palo Alto, California) under the provided instruction. All PCR inserts were examined with Nested Primer 1 and Nested Primer 2R on 2% Agarose/EtBr gels before the cDNA dot blot duplicate were prepared onto the positively-charged nylon membrane (Boehringer Mamheim, Indianapolis, Indiana) from either the forward or the reverse subtraction library for further library screening.

The cDNA dot blot duplicate from forward subtraction library were pre-hybridized with DIG Easy Hyb solution (Boehringer Mamheim, Indianapolis, Indiana) at 50°C for 1 hour and hybridized respectively with random-primed Dig-labeled probe prepared from forward subtraction library and reverse subtraction at same temperature for 14 hours. The same protocol was simultaneously applied to the duplicate from reverse subtraction library. Washing procedures and detection of Dig-labeled nucleic acids were standardized under the Genius™ System User's Guide for membrane hybridization (Boehringer Mamheim, Indianapolis, Indiana). By observing the differential signals present in dot blot duplicates, 26 positive clones were screened from forward subtraction library and 14 from reverse subtraction library. Plasmids with positive inserts were prepared with Plasmid Midi Kit (Qiagen, Chatsworth, California). These inserts were sequenced with dGTP mix by using Thermo Sequenase ^{33}P radiolabeled terminator cycle sequencing Kit (Amersham, Cleveland, Ohio). All sequences were applied to BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) so that they could be determined whether a known sequence was identified at both nucleic acid and amino acid levels by its alignment to the DNA and protein databases.

Among the 26 clones from the forward subtraction library, 25 were homologous to known genes, as follows:

Gene	Number
glucosoephosphate isomerase and neuroleukin	1
pyruvate kinase	1
heme oxygenase	1
ubiquitin/ribosomal fusion protein	1
alpha-enolase	2
proteasome theta chain	2
G119K BGH mutant	17

One clone was considered irrelevant.

Among 14 clones from the reverse subtraction library, ten were homologous to known genes, as follows:

Gene	Number
trans-Golgi network protein	1
medium chain acyl-CoA dehydrogenase	1
adipocyte lipid binding protein	2
mitochondrial cytochrome c oxidase	1
NADF1-ubiquonone oxidoreductase	2
cytochrome b	3

There were also four novel sequences: Ng-G119K2, Ng-G119K15, Ng-G119K36 and Ng-G119K62. Two of these were further studied in Ex. 2.

Example 2

Brown Adipose Tissue (BAT) Total RNA Preparation

70-day old male G119K growth hormone antagonist (GHA) mice and their non-transgenic (NT) littermates were sacrificed by neck dislocation. Their interscapular brown adipose tissues (BAT) were immediately dissected, weighed, placed in 10 volumes of cold RNA STAT-60™ solution (TEL-TEST "B", Friendswood, Texas), and carefully homogenized on ice. Total RNAs were prepared by following the manufacturers protocol. Total RNA

pellets were usually stored in 75% ethanol at -80°C for not more than 6 months.

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(CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT) or adaptor 2R (CTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGT) under the direction of PCR-Select™ cDNA Subtraction Kit so that GHA-adaptor 1, GHA-adaptor 2R, NT-adaptor 1, and NT-adaptor 2R were respectively prepared to serve as experimental tester cDNAs while unligated *Rsa* I-restricted cDNAs were used as experimental driver cDNAs.

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The cDNA dot blot duplicate from forward subtraction library were pre-hybridized with DIG Easy Hyb solution

(Boehringer Mamheim, Indianapolis, Indiana) at 50°C for 1 hour and hybridized respectively with random-primed Dig-labeled probe prepared from forward subtraction library and reverse subtraction at same temperature for 14 hours. The same protocol was simultaneously applied to the duplicate from reverse subtraction library. Washing procedures and detection of Dig-labeled nucleic acids were standardized under the Genius™ System UserÕs Guide for membrane hybridization (Boehringer Mamheim, Indianapolis, Indiana). By observing the differential signals present in dot blot duplicates, 26 positive clones were screened from forward subtraction library and 14 from reverse subtraction library. Plasmids with positive inserts were prepared with Plasmid Midi Kit (Qiagen, Chatsworth, California). These inserts were sequenced with dGTP mix by using Thermo Sequenase ^{33}P radiolabeled terminator cycle sequencing Kit (Amersham, Cleveland, Ohio). All sequences were applied to BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) so that they could be determined whether a known sequence was identified at both nucleic acid and amino acid levels by its alignment to the DNA and protein databases. Based on these internet searches, two novel partial cDNA sequences from the reverse subtraction library, Ng-G119K36 and Ng-G119K62, have been chosen for further study.

SMART™ PCR cDNA Library Construction

NT BAT SMART™ PCR cDNAs were synthesized from fresh purified NT BAT total RNAs with CDS primer and SMART II oligonucleotide at PCR cycle 20 by using SMART™ PCR cDNA Library Construction Kit (CLONTECH, Palo Alto, California). The integrity of this SMART cDNA was examined with 5 pairs of primers: 540 bp mouse β-actin, 606 bp mouse uncoupling protein 1 (UCP_1), 521 bp mouse GHR, 452 bp mouse glycerol-3-phosphate dehydrogenase (G3PDH), and approximately 250 bp hypoxanthine phosphoribosyltransferase (HPRT). The PCR program was 1 cycle of 94°C for 2 minutes, 55 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 68~72°C for 1~2 minutes, 1 cycle of 68~72°C for 7 minutes, and held at 4°C.

The polished SMART ds cDNA were ligated with 5' overhang *Eco*R I adaptor (13-mer: 5'-OH-AATTCGGCACGAG-3'; 9-mer: 3'-GCCGTGCTC-Pi-5') by using the ZAP-cDNA® Gigapack® III Gold Cloning Kit (STRATAGEN, La Jolla, California), purified by organic reagent extraction, precipitated with NaAC and ethanol, and physphorylated in the presence of T4 polynucleotide kinase and ATP, then size-fractionated with column provided in this ZAP-cDNA® Gigapack® III Gold Cloning Kit. Fractional drops from number 12 through 18 were collected together and further precipitated with cold ethanol at Ð20°C overnight. 25~100 ng of this SMART cDNA were then ligated to 1 µg of 41 kb λZAPII predigested vector at 12°C overnight and packaged with Gigapack® III Gold packaging extract at 22°C for 1.5 hours.

Following the instruction of ZAP-cDNA® Gigapack® III Gold Cloning Kit, 200 µl of 0.5 OD_{600} fresh XL-1 Blue MRF^- strain was mixed with 0.1-1 µl of packaged SMART cDNAs in 2-3 ml of NZY top agar at 48~55°C, then immediately inoculated onto a fresh NZY agar plate (100-mm), and incubated at 37°C for 8 hours. The titer ranged from 1.5-1.8 X 10^6 plaque forming unit (pfu) every 1 µg vector arm. 600 µl of 0.5 OD_{600} fresh XL-1 Blue MRF^- strain was mixed with a twentieth aliquot of packaged SMART cDNA in 6.5 ml of NZY top agar at 48~55°C, then immediately inoculated onto a fresh NZY agar plate (150-mm), and incubated at 37°C for 8 hours. The titer was approximately 4.05 X 10^5 pfu per plate (150-mm). The amplified library was made by pooling all samples together with SM dilution buffer and stored in 7% (v/v) dimethylsulfoxide (DMSO) solution at Ð80°C. The titer was similarly determined on the NZY plate (100-mm): 4.30 X 10^5 pfu/µl amplified library. A mini-PCR reactions were set up as 3.25 µl dH_2O, 0.5 µl 10X PCR buffer, 0.05 µl 10 µM T_7 primer and 0.05 µl 10 µM $M_{13}R(-48)$ primer, 0.1 µl 10 mM dNTPs, 0.05 µl 5 unit/ml Taq DNA polymerase (Promega, Madison, Wisconsin), and 1.0 µl DNA template-containing released particle from every isolated plaque with the parameters: 1 cycle of 95C for 2 minutes, 50 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 5 minutes, 1 cycle of 72°C for 7 minutes, and 4°C to hold. All total 5 µl reaction volume per reaction was loaded to 1% Agarose/EtBr gel

to determine the recombinational rate.

SMART™ PCR cDNA Library Screening

600 µl of 0.5 OD_{600} fresh XL-1 Blue MRF^- strain was mixed with approximately 2 µl of amplified library in 6.5 ml of NZY top agar at 48~55°C, then immediately inoculated onto a fresh NZY agar plate (150-mm), and incubated at 37°C for 8 hours. Duplicates of pre-shrinked (sterilization cycle for 10 minutes) nylon membranes (132 mm Φ) (Boehringer Mamheim, Indianapolis, Indiana) were placed to pre-cooled plaques on NZY agar plates for 2 minutes to make the first duplicate and for 4 minutes to make the second duplicate, orientated with insoluble ink by needling at its edge through the NZY agar, then blotted onto the filter papers. These plaque lifts were immobilized when autoclaving at sterilization cycle for 2 minutes.

Two entire inserts of positive clone Ng-G119K36 and Ng-G119K62 from reverse subtraction library were used to prepare the probes in the presence of Nested Primer 1 and Nested Primer 2R with the PCR DIG Probe Synthesis Kit (Boehringer Mamheim, Indianapolis, Indiana). The concentration of both Ng-G119K36 and Ng-G119K62 probes were estimated with the series dilution of Dig-labeled marker.

The plaque lift duplicates were pre-hybridized with DIG Easy Hyb solution (Boehringer Mamheim, Indianapolis, Indiana) at 42°C for 1 hour and hybridized respectively with PCR Dig-labeled probe prepared from either clone Ng-G119K36 or clone Ng-G119K62 from reverse subtraction library for about 12 hours. Washing procedures and detection of Dig-labeled nucleic acids were standardized under the Genius™ System User's Guide for membrane hybridization (Boehringer Mamheim, Indianapolis, Indiana). Plaques showing signals in plaque lift duplicates were isolated and prepared for further multiple screening on NZY agar plate (100-mm) until plaques were purified.

Following the protocol provided with ZAP-cDNA® Gigapack® III Gold Cloning Kit (STRATAGEN, La Jolla, California), SOLR strain was prepared to excise the pBluescript phagemid out from each purified plaque screened from full-length NT BAT SMART cDNA library. Colonies were isolated from LB-ampicillin

plates, prepared with Plasmid Maxi Kit (Qiagen, Chatsworth, California), and maintained for further studies.

Inserts within purified plasmids were sequenced with either dITP mix or dGTP mix by using Thermo Sequenase ^{33}P radiolabeled terminator cycle sequencing Kit (Amersham, Cleveland, Ohio). Downstream sequence determined from each reaction was used to design the primer for next run sequencing reaction until all sequences from a single insert can be overlapped together to deduce a completed full-length cDNA sequence. All full-length sequences were applied to BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) so that they could be determined whether a known sequence was called at both nucleic acid and amino acid levels by its alignment to the DNA and protein databases. An automated DNA sequencing approach was also employed in this project as well. Half reactions followed by isopropanol precipitation were chosed by using ABI PRISM© BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, California). All samples were arranged to the ABI PRISM 310 Genetic Analyzer. Sequences done with automated fashion were edited with the ABI PRISM EditView 1.0.1.sea software which is downloaded from the website (http://www2.perkin-elmer.com/ab/techsupp/softlib/SeqAnal/installs/mac/EditView_1.0.1.sea.hqx).

In general, two novel cDNAs sequences were identified. Clone 42 was screened with a Ng-G119K36 PCR Dig-labeled probe. Clone 42 codes a 2478-bp mRNA with an open reading frame encoding 346-amino acid sequence. Clone 42 has two isoforms, 2.4-kb & 1.2-kb, which seem down-regulated in GHA mice. Clone 42 is widely expressed in most tissues; there are significant levels of both isoforms in BAT, a pronounced level of the long isoform in brain and a striking level of the short isoform in testis. Predicted secondary structure seems a helix-like polypeptide with a 18-amino acid signal peptide and relatively low hydrophobicity value. Predicted tertiary structure contains 7-8 hydrophobic regions through the sequence. The folding model seems similar to bacteriorhodopsin which is a important protein for proton conductance in archaebacterial.

A BLAST search (Fig. 2(e)) demonstrates that Clone 42 is highly homologous to Clone 25077 mRNA from human female fetal brain tissue and PTD 010 mRNA from human pituitary tumor at both mRNA level and amino acid level.

Three other clones, Clone 58 (1380 bp), Clone 65 (2437 bp), and Clone 66 (1613 bp) were screened with the same Ng-G119K62 PCR Dig-labeled probe.

Clone 58, 65, & 66 seem like triple alternative splice forms which are 1379-bp, 2436=bp, 1612-bp mRNAs respectively. All these isoforms may encode a possible open reading frame containing 86 amino acids. The predicted open reading frame has a homology of two human genomic DNA sequences from clone 415G2 on chromosome 22 which contains synapsin IIa exon 1, EST and GSS. All these isoforms do not match up with any sequence in public protein database. The expression of long isoform tend to be BAT-specific.

Conclusions

Body weight and subcutaneous adipose tissue seems accumulated with age in GHA mice. An impairment of GH-induced signaling leads to abnormal growth of interscapular adipose tissues. GH-induced signaling has also been observed to down-regulate uncoupling protein-1 at transcriptional level.

In interscapular brown adipose tissue, GH may down-regulate genes coding glycolytic enzymes, ubiquitin/proteasome degradation machinery, and heme oxygenase; GH may up-regulate genes coding adipocyte lipid binding protein, trans-Golgi network protein (TGN38), medium chain acyl-CoA dehydrogenase, and mitochondrial innermembrane proteins for electron respiration chain. These GH-regulatable genes may be used as potential molecular markers to help explain obesity in GHA mice.

Clone 42 codes a 2478-bp mRNA with an open reading frame encoding 346-amino acid sequence. Two isoforms, 1.2-kb & 2.4-kb, are up-regulatable by GH and are widely expressed in most tissues with significant levels in BAT, pronounced level of long isoform in brain, and striking level of the short isoform in testis. It seems a helix-like polypeptide with a 18-amino

acid signal peptide and relatively low hydrophobicity value. Clone 42 is highly homologous to Clone 25077 mRNA from human female fetal brain tissue and PTD 010 mRNA from human pituitary tumor at both mRNA level and amino acid level.

Clone 58, 65, & 66 seem like triple alternative splice forms which are 1379-bp, 2436-bp, 1612-bp mRNAs respectively. They may encode a possible open reading frame containing 86 amino acids, with a homology of two human genomic DNA sequences from clone 415G2 on chromosome 22 containing synapsin IIIa exon 1, EST and GSS. The expression of clone 65 tend to be BAT-specific.

Example 3

A. Use of Mouse BAT genes in Assay of Human BAT

Brown adipose tissues are obtained from the human subject in a conventional, medically acceptable manner. Total RNA is then extracted using mL RNAStat60 per gram of tissue.

To 15-20 ug of brown adipose tissue RNA isolates, 1X MOPS, formaldehyde, formamide and ethidium bromide will be added, heat denatured at 60 °C then loaded on a formamide containing denaturing 1% agarose gel. The RNA will then be resolved by electrophoresis at 50V for about 2-2½h. After electrophoresis, the gel will be washed twice briefly with deionized water; then once with 0.05N NaOH, with 0.1M Tris at pH 7.5, and with 10X SSC at washing times of at least 30 min in each case.

The resolved RNA after electrophoresis will be transferred onto a nylon membrane by upward gradient adsorption using 10X SSC as transfer buffer. The RNA on the membrane will be UV crosslinked at 120 mJ, after which the RNA blots will be ready for hybridization.

B. Northern Blot Hybridization involving Non-radioactive DIG-labeled probe

Northern blot hybridization using digoxigenin (DIG)-labeled probe will be conducted to determine whether the genes of interest are present in brown adipose tissue RNA blots. The probes to be used for hybridization will be prepared from pCR2 clones, which contain as inserts the fragments isolated by

subtractive hybridization of brown adipose tissue genes from GHA mice versus WT mice.

1. Preparation of DIG-labeled probe

The DIG-labelled probe preparation will require PCR amplification of the inserts in pCR2 clones using Taq polymerase as polymerization enzyme and pCR 2.1A and pCR 2B as primers. The conditions for PCR amplification will be 95°C for 2 min.; 55 cycles at three temperature conditions of 95°C for15 sec., 58°C for 20 sec., and 72 °C for 45 sec.; then 72°C for 7 min. The amplified double-stranded cDNA fragment will undergo a second PCR amplification using a single primer, pCR 2.1A, in the presence of DIG labeled dNTPs to produce a single stranded DIG-labeled PCR product which will serve as the probe for RNA blot hybridization. The concentrations of the DIG labeled probe will be determined by comparing the signals produced by the probe to that of control DIG-labeled DNA upon exposure to radiographic film.

2. RNA Blot Hybridization

The concentration of DIG-labeled probe to be used for hybridization will be 50ng/mL of DIG Easy Hyb solution (Boehringer-Mannheim). Prior to hybridization, the RNA blots will be prehybridized in DIG Easy Hyb solution at 42 °C for 30-60 min. Following prehybridization, the RNA blots will undergo hybridization using the probes prepared form the different pCR 2 clones. Hybridization will be done at 42 °C for at least 8 hours.

Posthybridization washings of the membrane will then be performed at room temperature for 5min using a solution of 2X SSC and 0.1% SDS; and twice at 60 °C for 15 min. using a solution of 0.5X SSC and 0.1%SDS. The RNA blots will then be incubated with DIG antibody, which is conjugated to alkaline phosphatase. This antibody recognizes the DIG labeled hybrids in the RNA blot. CSPD (Boehringer-Mannheim), which is a chemiluminescent substrate for alkaline phosphatase, will be use to achieve detection of the RNA of interest in the blot. The presence of bands that is specific to the brown adipose

tissue genes of interest could be diagnostic of brown adipose tissue damage.

C. Northern Blot Hybridization involving ^{32}P-labeled probe

1. Preparation of ^{32}P-labeled probe

The ^{32}P-labeled probe will be prepared by first isolating the cDNA fragments that were inserted into the pCR 2 vector by performing EcoRI restriction enzyme digestion. The fragments will be purified though a Qiaex^{R} agarose gel extraction column (Qiagen). A 25ng of the purified fragment will serve as a template for the production of single-stranded ^{32}P-labeled probe using Random Primed DNA Labeling kit (Boehringer-Mannheim). The unincorporated dNTPs will be separated from the radiolabeled fragments using STE Select D G-25 column. The purified radiolabeld probe will then be quantified to determine the activity of the probe per ug of the DNA template. A good labeling of the template would have a specific activity range of 10^{8}-10^{9} cpm/ug of the template DNA.

2. RNA Blot hybridization

Prior to hybridization, prehybridization of the RNA blots will be performed by incubating the membrane in prehybridization solution made up of 50% formamide, 1% SDS, 1M NaCl, and 10% Dextran sulfate for 1 hour at 42 °C. Hybridization of the RNA blot with the ^{32}P-labeled probes prepared will follow after prehybridization. This will be conducted at 42 °C for at least 8 hours. Washing of the blots will be conducted once with 2X SSC at room temp for 5 min. and then with 2X SSC, 0.1% SDS at 56 °C which could last for about 5 minutes to an hour depending on the intensity of the radiactive signal. Radiographic exposure of the blots will determine whether the genes of interest are present.

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All patents or publications cited anywhere in this specification are hereby incorporated by reference in their entirety.

Table A

Human Genes regulated by Growth hormone (GH) and its antagonist in Brown Adipose Tissue

	Gene	DNA ID	Protein ID	GHA	NT	GH
1	neuroleukin	N/A	N/A	+		↓
	gucosephosphate isomerase	NM000175	NP000166	+		↓
2	α-enolase	X84907	NP001419	+		↓
3	pyruvate kinase	NM002654	A33983/S64635	+		↓
4	proteasome 0 chain	NM002795	NP002786	+		↓
5	heme oxygenase	D21243	NP002125/P30519	+		↓
6	Ubiquitin/ ribosomal fusion protein	NM00333	NP003324	+		↓
7	trans-Golgi network 38	N/A	N/A		+	↑
8	adipocyte lipid binding protein	NP001442/J 02874	NP001433		+	↑
9	medium chain acyl-CoA dehydrogenase	U07159	N/A		+	↑
10	NADH-ubiquonone oxidoreductase	V00711	P03905/CAA24035		+	↑
11	cytochrome b	V00711	AAC28269-88		+	↑
12	cytochrome c oxidase	V00711	BAA07292		+	↑
13	Ng-G119K2 (Novel)				+	↑
14	Ng-G119K15 (Novel)				+	↑
15	Ng-G119K36 (Novel)				+	↑
16	Ng-G119K62 (Novel)				+	↑

GHA: present in GH antagonist mouse cDNA substraction library (forward)

NT: present in nontransgenic mouse cDNA substraction library (reverse)

GH: presumed regulatory effect of GH on gene expression

Table B Identification of Homologous Mouse/Rat Genes and Proteins

Genes	DNA Accession #	Protein Accession #	Clone #s
glucosephosphate isomerase & neuroleukin	U89408 & M14220	P06745	44
a-enolase	X52379	P17182	27, 141
pyruvate kinase	X97047	CAA65761	12
proteasome theta chain	D21800 (rat)	P40112 (rat)	19, 59
heme oxygenase	AF029874	2984774 or 3169816	128
Ubiquitin/ribosomal fusion protein	AF118402	S11248	30
trans-Golgi network 38	D50031, D50032		68
adipocyte lipid binding protein	K02109 or M13385	1ALB or P04117	99, 23
medium chain acyl-CoA dehydrogenase	U07159		128
NADH-ubiquonone oxidoreductase	V00711	P03925	19
cytochrome b	V00711	CAA24092 or CAB09443	18
cytochrome c oxidase	V00711	P00397	45

Underlined genes were more strongly expressed in GHA mice, and hence are down-regulated by GH (negative markers). The remaining genes were more strongly expressed in normal mice than in GHA mice, and hence are up-regulated by GH (positive markers).

DNA and protein #s are for mouse unless otherwise stated.

Table D Summary of genes regulated by Growth Hormone (GH) and GH antagonist (GHA) in Brown Adipose Tissue (BAT)

	Message present in PCR-Select mouse cDNA subtraction libraries			
Identified Gene	GHA(25)		NT(14)	
	f	IVR	*f*	IVR
Bovine Growth Hormone	14	21.2-∞		
α-enolase/Neuroleukin	5	4.7-257.0		
Glucosphosphate Isomerase	1	3.9		
Pyruvate Kinase	1	8.4		
Ubiquitin/ribosomal Fusion Protein	1	11.2		
Proteasome θ Chain	2	35.4~78.7		
Heme Oxygenase	1	31.3		
Trans-Golgi Network Protein (TGN38)			1	2.4
Adipocyte Lipid Binding Protein			2	3.6-11.5
Medium Chain Scyl-CoA Dehydrogenase			1	31.1
NADH-ubiquonone Oxidoreductase			2	7.1-12.3
Cytochrome c Oxidase			2	1.9-5.2
Cytochrome b			2	1.8-2.3
Novel partial cDNAs			4	35.2-∞

Table D. Summary of genes regulated by Growth Hormone (GH) & GH antagonist (GHA) in Brown Adipose Tissue (BAT). In the table, "*f*" indicates number of positive clones, whereas "IVR" is abbreviated from Intensity Volume Ratio estimated from library screening Dot Blots. "GHA" represents the forward

subtraction library (subtracting NT mouse BAT cDNA from GHA's). 17 out of 26 sequences were found to be bGH G119K EST. 1 out of 26 was found to be a contaminant. "NT" represents the reverse subtraction library (subtracting GHA mouse BAT cDNA from NT's). 4 out of 14 were determined as novel ESTs after applying BLAST searches.

Results: Genes encoding glucosephosphate isomearse, α-enolase, pyruvate kinase, proteasome θ chain, ubiquitin, and heme oxygenase were found in the forward subtraction library, indicating that these genes are up-regulated in GHA mouse BAT. Genes encoding mitochondria cytochrome b, mitochondria cytochrome C oxidase subunit I, mitochondria NADH-ubiquinone oxidoreductase chain 4 and/or 6, medium chain acyl-CoA dehydrogenase, adipocyte lipid binding protein, and trans-Golgi network protein (TGN38) were found in the reverse subtraction library, indicating that these genes are down-regulated in GHA mouse BAT. All these GH-regulated genes may be used as potential molecular markers to help explain obesity in GHA mice.

Table 1 shows certain of the nucleotide sequences identified by subtractive hybridization against the mouse brown adipose tissue library. These are the sequences which appeared to be identical, or nearly identical, to a databank sequence. Presumably, the corresponding human genes are similarly regulated. The sequence names, sequence lengths, and the names of the most closely related databank sequences, are set forth below:

(A) G119K-Ng 44 (361 bp)(glucosephosphate isomerase; neuroleukin)

(B) G119K-Ng27 (550 bp) and G119K-Ng 141 (363 bp)(alpha-enolase)

(C) G119K-Ng12 (300 bp)(pyruvate kinase)

(D) G119K-Ng19 & 59 (299 bp)(proteasome theta chain)

(E) G119K-Ng128 (336 bp)(heme oxygenase)

(F) G119K-Ng30 (303 bp)(ubiquitin)

(G) G119K-Ng68 (345 bp)(trans-Golgi network protein)

(H) G119K-Ng99 (313 bp) and G119K-Ng123 (374 bp)(adipocyte lipid binding protein)

(I) G119K-Ng127 (542 bp)(medium chain acyl-CoA dehydrogenase)

(J) G119K-Ng19 (725 bp) and 160 (131 bp)(NADH-ubiquinone oxireductase)

(K) G119K-Ng45 (343 bp)(cytochrome c oxidase)

For each sequence, the complete clone sequence, and the highest scoring BLAST alignment, are given.

Table 2 (a) shows the sequences of Ng-G119K42, and (as boldfaced subsequence) Ng-G119K36. (B) shows ORF.

The ORF of clone 42 spanning from nucleotide 112 through 1152 is believed to encode a 346 amino acid long polypeptide, which possesses a predicted cleavage site most likely between residue Q18 and P19 based on the output of Signal V1.1 World Wide Web Server (http://www.cbs.dlu.dlk/services/SignalP/).

The clone 42 protein has a theoretical Molecular Weight of 37.2751 Kda, theoretical Isoelectric Point of 9.82, 97.05 in aliphatic index, and 0.382 in grand average of hydropathicity, by ProtParam tool (http://www.expasy.ch/tools/protparam.html).

The secondary structure prediction for clone 42 suggests an all-helices protein which contains 38.44% for α-helix, 22.54% for extended strand, 12.72% for β-turn, and 26.30% for random coil, by the SOPM method (http://pbil.ibcp.fr/egi-bin/npsa_sopm.html) [Geourjon, C. & Deléage, G., SOPM: a self optimised method for protein secondary structure prediction, Protein Engineering (1994) 7, 157-164]. By the SOPMA method (http://pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html), we get 51.73% for α-helix, 16.18% for extended strand, 5.49% for β-turn, and 26.59% for random coil.

The polypeptide encoded by clone 42 may have 6-8 transmembrane (TM) regions, based on the output from "DAS" - Transmembrane Prediction server (http://www.biokemi.su.se-server/DAS/). Depended on the criteria applied for prediction, several other free public servers suggest 6 TM, see TMHMM (v. 0.1) program (http://www.cbs.dtu.dk/services/TMHMM-1.0/), 7 TM by TopPred 2 program (http://www.biokemi.su.se/-server/toppred2/toppredServer.cgi), or 8 TM, see Tmpred program (http://www.ch.embnet.org/software/TMPRED_form.html).

The Tmpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of Tmbase, a database of naturally occurring transmembrane proteins. The prediction is made using a combination of several weight-matrices for scoring.

No suitable target has been found in searching sequences of known 3D structures from the SWISS-MODEL Protein Modelling Server (http:/www.expasy.ch/swissmod/SWISS-MODEL.hlml).

However, the protein folding encoded by clone 42 is suggested as being similar to a membrane and cell surface protein and peptide, such as bacteriorhodopsin (0.465 of SAWTED E-value and 0.364 of PSSM E-value) at low significant level, based on the Program 3D-PSSM (http://www.bmm.icnel.uk/-3dpssm/) which has a capacity to recognize protein fold using 1D and 3D sequence profiles coupled with secondary structure information (Foldfil).

Potential type O-glycosylation sites S186, T163, and T269 are predicted from NetOGlyc 2.0 Prediction Server (http://www.cbs.dlu.dk/services/NclOGlycl) which produces neural network predictions of mucin type GalNAc O-glycosylation sites in mammalian proteins. Potential phosphorylations sites at S71, S127, S182, T50, T54, T59, T306, T322, Y46, and Y183 are predicted from the NetPhos WWW server (http://www.cbs.dlu.dk/services/NetPhos/) which produces neural network predictions for serine, threomine and tyrosine phosphorylation sites in eukaryotic proteins.

Results: Predicted secondary structure of clone 42 seems a helix-like polypeptide with a 18-amino acid signal peptide and relatively low hydrophobicity value. Predicted tertiary structure of clone 42 is a protein with a 6-8 hydrophobic regions through the sequence, leading to a folding model similar to bacteriorhodopsin, which is a important protein for proton conductance in archaebacteria.

Applying the advanced version of Blast search with a controlled expect value below 0.0001, 12 bits have been found matchable to the sequence of clone 42 in positive sense strand at amino acid level. Among them, three sequences from Genebank database share a protein homogy at a very significant level as listed in this figure: C25077 gene product from female human infant brain with an expect value 1.0 $x10^{-170}$, PTD010 gene product from human pituitary tumor with an expect value 1.0 x 10^{-147}, and CG1287 gene product from drosophila melanogaster with an expect value 4.0 x 10^{-73}. The C25077 gene product from female human infant brain possesses a same size of deduced polypeptide as DERP2. Clone 42-encoded polypeptide shares 86% (2980aa out of 346-aa) identities and 90% (313-aa out of 346-

aa) positives to C25077 gene product, 74% (256-aa out of 346-aa) identities and 77% (268-aa out of 346-aa) positives to PTD010 gene product, and 40% (139-aa out of 346-aa) identities and 54% (188-aa out of 346-aa) positives to CG1287 gene product.

Results: Since the PTD010 is missing the C-terminal portion of sequence which remains intact in C25077 and Clone 42, it suggests that the C-terminal region may be important for normal biological function.

At the nucleic acid level, applying the advanced version of BLAST search with a controlled expect value below 0.0001, many genes have been found matchable beyond the nucleotide position 1794 in positive sense stand of clone 42. They span approximately 100-bp. However, a few of matched sequences exhibited similarity from 5' end through most part of clone 42 with a zero expect value, such as C25077 gene (345-aa) of female human infant brain, PTD010 gene product from human pituitary tumor, and human dermal papilla derived protein 2 gene (DERP2). DERP2 is identical to C25077. Along with their matched portion, clone 42 shares 90% homology with both sequences, 946-bp out of 1049-bp for C25077 or 945-bp out of 1049-bp for PTD010.

Table 3 shows the sequences of (a) Ng-G119K58, (b) Ng-G119K65, (c) Ng-G119K66, and the alignment of EST NG-G119K62 with each of (a)-(c). 3(d) shows the triple alignment of (a), (b) and (c). 3(e) shows ORF of greatest interest.

Clones 58, 65, and 66 are 1379-bp, 2437-bp, and 1613-bp long mRNA respectively and have 3 ORFs, 7 ORFs, and 3 ORFs respectively. 5 out of total 9 OFRs are derived from positive strand of cDNAs: 1 specific to clone 65, 2 specific to clone 65 and 66, and 2 specific to clone 58, 65 and 66. The sequence of Reverse 62 completely matches all these three clones at 3' end which is upstream of the multiple polyadenylational tail signals (AAUAAA). Additional polyadenylational signal sequence is unusually seen in 5' end of clone 65, but whole context suggests it may not functional.

Clone 58, 65, and 66 seem like triple alternative splice

forms which are 1379-bp, 2436-bp, 1612-bp mRNAs respectively, which support the prediction by sequence analyses of these clones. Because multiple bands were observed under prolonged exposure, other unknown spliced forms may exist in this gene family. Messages from ORF region encoded multiple sequences and clone 65 itself seem negatively regulated by GH at transcriptional level in mouse BAT.

Applying the advanced version of Blast search with a controlled expect value below 0.000.1, many genes have been found matchable beyond the nucleotide position 1203 for clone 58, 1856 for clone 65, and 1437 for clone 66 in antisense strand for these cDNAs. However, in positive sense strand, only human DNA sequence from clone 415G2 on chromosome has been matched to clone 58 with an expect value 3.0×10^{-50}, to clone 65 with an expect value 6.0×10^{-50}, and to clone 66 with an expect value 4.0×10^{-50} as indicated in A and B. These alignment searches suggest that all these three isoforms share a common open reading frame which encodes 86 amino acids.

Table 4 shows the sequences of (a) Ng-G119K2, first strand and second strand and (b) Ng-G119K15 first strand and second strand.

Applying the advanced version of BLAST search with a controlled expect value below 0.0001, the polypeptide sequence of clone 58 shares 49% (42-aa out of 86-aa) identities, 77% (66-aa out of 86-aa) positives with CG6115 gene product of Drosophila melanogaster with an expect value 1.0×10^{-16} for clone 58 and 2.0×10^{-16} for both clone 65 and 66, though there is no similarity at nucleic acid sequence level. This output further supports the prediction of ORF. Additionally, DNA sequence homology only occurs between human and mice, not between mice and fruit fly, suggesting a evolutionary role within these species.

Table 5 shows the sequence of Ng62D'4-2-1-4 cDNA.

Table 1

mouse glucosephosphate isomerase & neuroleukin partial (+) strand cDNA Open Reading Frame

G119K-Ng 44 sequence (361 bp):

CTCTTTATAATCGCCTCCAAGACCTTCACCACCCAGGAGACCATCACCAATGCAGAGACA
GCAAAGGATGGTTTCTCGAAGCGGCCAAGATCCATCTGCAGTTGCAAAGCACTTTGTCGC
CCTGTCTACGAACACGGCCAAAGTGAAAGAGTTTGGAATTGACCCTCAAAACATGTTCGA
GTTCTGGGATTGGGTAGGTGGCCTATTCGCTGTGGTCAGCCATTGGACTTTCCATTGCTC
TGTATGTAGGTTTTGACCACTTCGAGCAGCTGCTGTCCGGGGCTCACTGGATGGACCTGC
ACTTCCTCAAGACGCCCCTGGAGAAGAATGCCCCCGTCCTGCTGGCTCTACTGGGCATCT
G

Sequence 1 1c11G119k-Ng 44 **Length** 361 from:1 to = 361

Sequence 2 gi 200064 Mouse neuroleukin mRNA, complete cds.
Length 1985 from: 1 to +1985

NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 639 bits (332), Expect = 0.0
Identities = 359/365 (98%), Positives = 359/365 (98%), Gaps = 4/365 (1%)
Aligned query 1-361 to subject 665-1029.

Sequence 1 G119K-Ng 44 **Length** 361 from:1 to = 361

Sequence 2 gi 3642648 Mus musculus strain BALB/c glucosc-6-phosphate isomerase mRNA, partial cds.
Length 477 from:1 to = 477

Score = 462 bits (240), Expect = e-128
Identities = 265/275 (96%), Positives = 265/275 (96%), Gaps = 2/275 (0%)
Aligned query 89-361 to subject 1-275.

2. mouse α-enolase

partial (+) strand cDNA Open Reading Frame

G119K-Ng 27 sequence (550 bp):

ACGCGGAAGCAGTGGTAACAACGCAGAGTACACCGCAAAAGGTCTCTTCCG
AGCTGCGGTGCCCAGCGGTGCGTCCACTGGCATCTACGAGGCCTAGAACTC
CGAGACAATGATAAGACCCGCTTCATGGGGGAAGGGTGTCTCACAGGCTGTT
GAGCACATCAATAAAACTATTGCGCCTGCTCTGGTTAGCAAGAAAGTGAATG
TTGTGGAGCAAGAGAAGATTGACAAGCTGATGATCGAGATGGACGGCACAGA
GAATAAATCTAAATTTGGTGCAAATGCCATCCTGGGAGTGTCCCTGGCTGTC
TGCAAAGCTGGTGCCGTGGAAAAGGGGTGCCCCTTTACCGCCACATTGCTGA
CTTGGCCGGCAACCCTGAAGTCATCCTGCCTGTCCCGGCTTTCAATGTGATC
AACGGTGGTTCTCATGCTGGCAACAAAGCTGGCCATGCAAAGAGTTCATGAT
CCTGCCTGTGGGGCATCCAGCTCCGGGAAGCCATGCGCATTGGAGCAGAGGT
TTACCACAACCTGAAGAACGTGATCAAGGAGA

Sequence 1 G119K-Ng 27 **Length** 550 from:1 to = 550

Sequence 2 gi 55490 Mouse mRNA for alpha-enolase (2-phospho-D-glycerate hydrolase) (EC 4.2.1.11)
Length 1720 from:1 to = 1720

Score = 906 bits (471), Expect = 0.0
Identities = 520/527 (98%), Positives = 520/527 (98%), Gaps = 7/527 (1%)
Aligned query 28-550 to subject 167-690.

G119K-Ng 141 sequence (363 bp):

ACAAGTCCTTCGTCCAGAACTACCCAGTGGTGTCCATCGAAGATCCCTTTGA
CCAGGACGACTGGGGCGCCTGGCAGAAGTTCACGGCTAGTGCGGGCATCCAG
GTGGTGGGCGATGACCTCACAGTGACCAACCCTAAGCGGATTGCCAAGGCTG
CGAGCGAGAAGTCCTGCAACTGCCTCTTGCTCAAAGTGAACCAGATCGGCTC
TGTGACCGAATCCCTGCAGGCGTGTAAGCTGGCCCAATCCAATGGCTGGGGT
GTCATGGTGTCCCACCGATCTGGGGAAACTGAGGACACTTTCATCGCAGACC
TGGTGGTGGGGCTCTGCACTGGGCAGATCAAGACTGGTGCCCCTTGCCGAT

Sequence 1 G1119K-Ng 141 **Length** 363 from:1 to = 363

Sequence 2 gi 55490 Mouse mRNA for alpha-enolase (2-phospho-D-glycerate hydrolase) (EC 4.2.1.11)
Length 1720 from:1 to = 1720

Score = 675 bits (351), Expect = 0.0
Identities = 359/363 (98%), Positives = 359/363 (98%)
Aligned query 1-360 to subject 934-1293.

3. mouse pyruvate kinase
partial (+) strand cDNA Open Reading Frame

G119K-Ng 12 sequence (300 bp):

CATGCAGAGACCATCAAGAATGTCCGTGAAGCCACAGAAAGCTTTGCATCTG
ATCCCATTCTCTACCGTCCTGTTGTGGTGGCTCTGGATACAAAGGGACCTGA
GATCCGGACTGGACTCATCAAGGGCAGCGGCACCGCTGAGGTGGAGCTGAAG
AAGGGAGCCACTCTGAAGATCACCCTGGACAAGCTTACATGGAGAAGTGTGA
CGAGAACATCCTGTGGCTGGACTACAGACATCTGCAAGGTGTGAGTGGCAGC
AAGATCTACGTGGACGATGGCTCATCTCACTGCAGTGAAG

Sequence 1 G119K-Ng 12 **Length** 300 from:1 to = 300

Sequence 2 gi 1405932 M.musculus mRNA from M2-type pyruvate kinase
Length 2134 from:1 to = 2134

Score = 465 (242), Expect = e-129
Identities = 291/303 (96%), Positives = 291/303 (96%), Gaps = 8/303 (2%)
Aligned query 1-295 to subject 256-558.

4. mouse proteasome θ chain
partial (+) strand cDNA Open Reading Frame

G119K-Ng 19 & 59 sequence (299 bp):

GCGGGGACTCCAGCGCAATCATGTCTATTATGTCCTATAATGGAGGGGCCGT
CATGGCATGAAGGGAAAGAACTGTGTGGCCATCGCTGCAGACAGACGTTTCG
GGATCCAGGCCCAGATGGTGACCACGGACTTCCAGAAGATCTTTCCCATGGG

TGACAGGCTCTACATAGGCCTGGCCGCCTGGCCACTGACGTCCAGACAGTTG
CCCAGCGTCTCAAGTTCCGACTGAACTTGTATGAGCTGAAGAAGGTCGACAG
ATCAGCCTTACACCTACTGAGACTGGTGGCACTCTGTAT

Sequence 1 G119K-Ng 19 & 59 **Length** 299 from:1 to = 299

Sequence 2 gi 458730 Rat mRNA for proteasome subunit RC10-II, complete cds.
Length 828 from:1 to = 828

Score = 402 bits (209), Expect = e-110
Identities = 255/268 (95%), Positives = 255/268 (95%), Gaps = 4/268 (1%)
Aligned query 11-274 to subject 68-335.

5. mouse heme oxygenase
partial (+) strand cDNA Open Reading Frame

G119K-Ng 128 sequence (336 bp):
TCCAGTTGTCAAGACTTCTTGAAGGAAACATTAAGAAGGAGCTATTTAAGAT
GGCACCACTGCACTTTACTTCACATACTCAGCCCTTGAGAGGAATGGACCGC
AACAAGGACACCCAGCCTTCGCCCCCTTATATTTCCCCACGGAGCTACACCG
GAAGGCAGCACTGATCAAGGACATGAAGTATTTCTTTGGTGAAAACTGGGAG
GAGCAGGTGAAGTGCTCTGAGGCTGCCCAGAAGTATGTGGATCGGATTCACT
ATGTAGGGCAAAATGAGCCAGAGCTGCTGGTGGCCCATGCTTATACTCGTTA
CATGGGGGACTTTCAGGGGGTTAG

Sequence 1 G119K-Ng 128 **Length** 336 from:1 to = 336

Sequence 2 gi 2984773 **Length** 1255 from:1 to = 1255

Score = 527 bits (274), Expect = e-148
Identities = 311/317 (98%), Positives = 311/317 (98%), Gaps = 5/317 (1%)
Aligned query 22-303 to subject 1-284.

6. mouse ubiquitin
partial (+) strand cDNA Open Reading Frame

G119K-Ng 30 sequence (303 bp):

GGGCTTTCTCTTCAACGAGGCGGCCGAGCGGCAGACGCCAACATGCAGATCT
TCGTGAAGACCTGACGGGCAAGACCATCACTCTTGAGGTCGAGCCCAGTGAC
ACCATCGAGAATGTCAAGGCCAAGATCCAAGACAAGGAAGGCATCCCACCTG
ACCAGCAGAGGCTGATATTCGCGGGCAAACAGCTGGAGGATGGCCGCACCCT
GTCCGACTACAACATCCAGAAAGAGTCCACCTTCGACCTGGTGCTGCGTCTG
CGCGGTGGCATCATTGAGCCATCCTTCGTCAGCTTGCCCAGAA

Sequence 1 G119K-Ng 30 **Length** 303 from:1 to = 303

Sequence 2 gi 4262554 **Length** 500 from:1 to = 500

Score = 504 bits (262), Expect = e-141
Identities = 280/284 (98%), Positives = 280/284 (98%), Gaps = 2/284 (0%)
Aligned query 22-303 to subject 1-284.

7. mouse Trans-Golgi Network protein (TF_GN38) partial (+) strand cDNA

Ng-G119K 68 sequence (345 bp):

TAGCATAAAAGGGACTCGAGGTTTCTGAAAGTAAAATCACTGTTTGATGGGA
TTTTTTAAAAAAATGATCATTGAACAAGTGTGTTCTTGCATACATTCACCCC
AATAAGGGCTTCCTGGAAAGGGACAGGTTCATGCTTTGTGGAAGAAAACACA
TAGGAGGGATTTAGTATGCAGGAAAGAGGTTTTCTACAAATTGAGTTTTGCT
TTTATTGCCCGCAGTAGATAGATATTTAGAAACTAACTGCATTCTTCACACT
CCTCCTTGCTGTTTAAGATGTGCAGGATAGGAAATCTTCCTATCCTGTCATA
TCTGGTCATGAACTGTAGAACTAATAGTCCTGA

Sequence 1 Ng-G119K 68 **Length** 345 from:1 to = 345

Sequence 2 gi 949828 Mouse mRNA for TGN38A, complete cds.
Length 1673 from:1 to = 1673

Score = 89.1 bits (46), Expect = 3e-16
Identities = 48/49 (97%), Positives = 48/49 (97%)
Aligned query 1-49 to subject 1625-1673.

Sequence 1 Ng-G119K 68 **Length** 345 from:1 to = 345

Sequence 2 gi 949830 Mouse mRNA for TGN38B, complete cds.
Length 2265 from:1 to = 2265

Score = 487 bits (253), Expect = e-136
Identities = 308/329 (93%), Positives = 308/329 (93%), Gaps = 6/329 (1%)
Aligned query 1-324 to subject 1656-1983.

8. mouse adipocyte lipid binding protein partial (+) strand cDNA Open Reading Frames

Ng-G119K 99 sequence (313 bp):

GCAGAAGTGGGATGGAAAGTCGACCACAATAAAGAGAAAACGAGATGGTGAC
AAGCTGGTGGTGGAATGTGTTATGAAAGGCGTGACTTCCACAAGAGTTTATG
AAAGGGCATGAGCCAAAGGAAGAGGCCTGGATGGAAATTTGCATCAAACACT
ACAATAGTCAGTCGGATTTATTGTTTTTTTTAAAGATATGATTTTCCACTAA
TAAGCAAGCAATTAATTTTTTCTGAAGATGCATTTTATTGGATATGGTTATG
TTGATTAAATAAAACCTTTTTAGACTCAAAAAAAAAAAAAAAAAAAAAAGG
T

Sequence 1 Ng-G119K 99 **Length** 313 from:1 to = 313

Sequence 2 gi 198716 Mouse 3T3-L1 lipid binding protein mRNA, complete cds.
Length 614 from:1 to = 614

Score = 519 bits (270), Expect = e-146
Identities = 278/286 (97%), Positives = 278/286 (97%)
Aligned query 1-286 to subject 328-613.

Sequence 1 G119K-Ng 99 **Length** 313 from:1 to = 313

Sequence 2 gi 198718 Mouse adipocyte lipid binding protein gene, complete cds.
Length 5212 from:1 to = 5212

Score = 333 bits (173), Expect = 9e-90
Identities = 211/224 (94%), Positives = 211/224 (94%), Gaps = 4/224 (1%)

Aligned query 64-286 to subject 4490-4710.

Score = 114 bits (59), Expect = 8e-24
Identities = 63/65 (96%), Positives = 63/65 (96%)
Aligned query 1-65 to subject 3760-3824.

Ng-G119K 123 sequence (374 bp):

GAATTCGATGAAATCACCGCAGACGACAGGAAGGTGAAGAGCATCATAACCC
TAGATGGCGGGGCCCTGGTGCAGGTGCAGAAGTGGGATGGAAAGTCGACCAC
AATAAAGAGAAAACGAGATGGTGACAAGCTGGTGGTGGAATGTGTTATGAAA
GGCGTGACTTCCACAAGAGTTTATGAAAGGGCATGAGCCAAAGGAAGAGGCC
TGGATGGAAATTTGCATCAAACACTACAATAGTCAGTCGGATTTATTGTTTT
TTTTTAAAGATATGATTTTCCACTAATAAGCAAGCAATTAATTTTTTCTGAA
GATGCATTTTATTGGATATGGTTATGTTGATTAAATAAAACCTTTTTAGACT
CAAAAAAAAA

Sequence 1 Ng-G119K 123 **Length** 374 from:1 to = 374

Sequence 2 gi 198716 Mouse 3T3-L1 lipid binding protein mRNA, complete cds.
Length 614 from:1 to = 614

Score = 654 bits (340), Expect = 0.0
Identities = 355/364 (97%), Positives = 355/364 (97%), Gaps = 1/364 (0%)
Aligned query 1-364 to subject 251-613.

Sequence 1 Ng-G119K 123 **Length** 374 from:1 to = 374

Sequence 2 gi 198718 Mouse adipocyte lipid protein gene, complete cds.
Length 5212 from:1 to = 5212

Score = 344 bits (179), Expect = 4e-93
Identities = 211/224 (94%), Positives = 211/224 (94%), Gaps = 3/224 (1%)
Aligned query 141-364 to subject 4490-4710.

Score = 191 bits (99), Expect = 7e-47
Identities = 103/105 (98%), Positives = 103/105 (98%)
Aligned query 38-142 to subject 3720-3824.

Score = 75.7 bits (39), Expect = 4e-12

Identities = 39/39 (100%), Positives = 39/39 (100%)
Aligned query 1-39 to subject 3097-3135.

9. mouse medium chain acyl-CoA dehydrogenase partial (+) strand cDNA

Ng-G119K 127 sequence (542 bp):

GAGGCTGATCATAGCTCGTGAGCACATTGAAAAGTATAAAAATTAACAGGA
ATTACTATTGAACGATGCATCACCCTCGTGTAACTAAGCTCCAAGCACTGT
TGCTGCTTCAGGGGAAAAGGGCTTTACTGTCTTCCCAAGGAAATGAGATCAA
AGACGAGTTTGGATCTGTGCAGCGGATTCCCATGGCGGAGGAACCTGTCTTC
AGCTCTATGGTGACCCTTTCTAGATAGGTTTGGCTTTTGGACAATGATTGGT
CCTTAGCCCCGAATTGTGTTAGTTTGCTCTTTGATCACTTAAAATGGAAAAA
CACCCTGGACTTTTAATGTTCATTCAAGTGACAGGAAAGGCGGCTTGTCAAG
GAAGAACTCATGATTCTAACATAAACACTGAAAATTTGTGGTAGATTGGACA
CGTCAGACTGTGACATAGCAGCATTTCTGTGCTGAACTGTTAATTTTATAAT
TTTGATTATATTTGCTTTGTTTTGCACAAAAGAGTAAAAAGTTTATATTCAC
ATTCTCCCATTATAAAACTAAAAC

Sequence 1 Ng-G119K 127 **Length** 542 from:1 to = 542

Sequence 2 gi 463908 Mus musculus medium-chain acyl-CoA dehydrogenase mRNA, complete cds.
Length 1846 from:1 to = 1846

Score = 1021 bits (531), Expect = 0.0
Identities = 540/542 (99%), Positives = 540/542 (99%), Gaps = 1/542 (0%)
Aligned query 1-542 to subject 1228-1768.

10. mouse NADH-ubiquonone oxidoreductase partial (+) strand cDNA Open Reading Frame from mitochondria genome

Ng-G119K 19 sequence (725 bp): chain 4

GATCCGTTCGTAGTTGGAGTTTGCTAGGCAGAATAGGAGTGATGATGTGAG

GCCATGTGCGATTATTAGTATTGTTGCTCCTATGAAGCTTCATGGAGTTTG
GATTATGATTGATGCAATAACAAGTGCTATGTGGCTAACTGAGGAGTAGGC
GATTAGTGATTTTAAATCTGTTTGGCGTACAGAGATTGAGCTAGTTATAAT
TATTCCTCATAGGGAGAGAAGGATGAAGGGGTATGCTATATATTTTGTTAG
TGGGTCTAGAATAATGGAGATGCGAATTATTCCGTAACTACCTAATTTTAG
AAGAATAGCTGCTAGAATTATTGACCCAGCAATTGGAGCTTCAACATGGGCT
TTTGGTAGTCATAGGTGAACTCCATATAATGGTATTTTAATAAGAAATGCTA
TTATGCATGCCAACCATAGTAAGTTGTTAGATCATGAAGCGTCTAAGGTGTG
TGTTGTGAATGATAAAATTATGAGGTTTAGGGTTCCTACATGGTTTTGGATT
AAGATGAGGGCAATTAGCAGTGGAATAGAACCGATTAGGGTATAAAATAGGA
AATAAATCCCTGCGTTTAGGCGTTCAGTTTGGTTCCTCATCGGGTAATAATA
ATAAGTGTTGGGATTAAGGTTGCTTCAAATAAAATATAAAATATAATTAGTT
CAGTTGCTGAAAAGGTTATGATTAGGAGAATTTGTAAGCTGATTAGTATTGA
GAT

Sequence 1 Ng-G119K 19 **Length** 725 from:1 to = 725

Sequence 2 gi 13838 Mus musculus mitochondrial genome
Length 16295 from:1 to = 16295

Score = 1363 bits (709), Expect = 0.0
Identities = 722/726 (99%), Positives = 722/726 (99%), Gaps = 1/726 (0%)
Aligned query 1-725 to subject 11171-10446.

Ng-G119K 160 sequence (131 bp): chain 6

ATACTCAATTAATCTCGAGTAATCTCGATAATAATAAAAGATACCCGCAAAC
AAAGATCACCCAGCTACTACCATCATTCAAGTAGCACAACTATATATTGCCG
CTACCCCAATCCCTCCTTCCAACATAA

Sequence 1 Ng-G119K 160 **Length** 131 from:1 to = 131

Sequence 2 gi 13838 Mus musculus mitochondrial genome.
Length 16295 from:1 to = 16295

Score = 237 bits (123), Expect = 3e-61
Identities = 130/131 (99%), Positives = 130/131 (99%), Gaps = 1/131 (0%)
Aligned query 1-131 to subject 13538-13667.

11. mouse cytochrome b
partial (+) strand cDNA Open Reading Frame from mitochondria genome

Ng-G119K 18 sequence (599 bp):

GAGTCATAGCCACAGCATTTATAGGCTACGTCCTTCCATGAGGACAAATAT
CATTCTGAGGTGCCACAGTTATTACAAACCTCCTATCAGCCATCCCATATA
TTGGAACAACCCTAGTCGAATGAATTTCAGGGGGCTTCTCAGTAGACAAAG
CCACCTTGACCCGATTCTTCGCTTTCCACTTCATCTTACCATTTATTATCGC
GGCCCTAGCAATCGTTCACCTCCTCTTCCTCCACGAAACAGGATCAAACAAC
CCAACAGGATTAAACTCAGATGCAGATAAAATTCCATTTCACCCCTACTATA
CAATCAAAGATATCCTAGGTATCCTAATCATATTCTTAATTCTCATAACCCT
AGTATTATTTTTCCCAGACATACTAGGAGACCCAGACAACTACATACCAGCT
AATCCACTAAACACCCCACCCCATATTAAACCCGAATGATATTTCCTATTTG
CATACGCCATTCTACGCTCAATCCCCAATAAACTAGGAGGTGTCCTAGCCTT
AATCTTATCTATCCTAATTTTAGTCCTAATACCTTTCCTTCATACCTCAAAG
CAACGAAGCCTAATATTCCGCCCAATCACA

Sequence 1 Ng-G119K 18 **Length** 599 from:1 to = 599

Sequence 2 gi 13838 Mus musculus mitochondrial genome.
Length 16295 from:1 to = 16295

Score = 1138 bits (592), Expect = 0.0
Identities = 596/598 (99%), Positives = 596/598 (99%)
Aligned query 2-599 to subject 14504-15101.

12. mouse cytochrome c oxidase
partial (+) strand cDNA Open Reading Frame from mitochondria genome

Ng-G119K 45 sequence (343 bp):

TCCAGCTATACTATGAGCCTTAGGCTTATTTCTATTACAGTTGGTGGTCTAA
CCGGAATGTTTATCCAACTCATCCCTTGACATCGTGCTTCACGATAACATAC

TATGTAGTAGCCCATTTCCACTATGTTCTATCAATGGGAGCAGTGTTTGCTA
TCATAGCAGGATTTGTTCACTGATTCCCATTATTTTCAGGCTTCACCCTAGA
TGACACATGAGCAAAAGCCCACTTCGCCATCATATTCGTAGGAGTAAACATA
ACATTCTTCCCTCAACATTTCCTGGGCTTTCAGGAATACCACGACGCTACTC
AGACTACCCAGATGCAAAAAAAAAAAAAAAA

Sequence 1 1c11seq_1 **Length** 343 from:1 to = 343

Sequence 2 gi 13838 Mus musculus mitochondrial genome.
Length 16295 from:1 to = 16295

Score = 531 bits (276), Expect = e-149
Identities = 323/334 (96%), Positives = 323/334 (96%), Gaps = 8/334 (2%)
Aligned query 1-327 to subject 6332-6664.

Table 2A

mouse Brown Adipose Tissue Reverse (NT-GHA) 36 cDNA (EST); 463 BP. (in bold)

mouse Brown Adipose Tissue Clone 42 cDNA (full-length); 2475 BP.

Start Codon: 112 (underlined)

Stop Codon: 1152

Open Reading Frame: 346-aa

GAGAGGGAGG TCGCACACTC TGAGTTTCGG TGACCCGGAA GGAGCCCCGT
GGTAGAGGTG ACCGGAGCTG AGCATTTCAG ATCTGCTTAG TAAACCGGTG
TATCGCCCAC CATGTTGGCT GCAAGGCTTG TGTGTCTCCG GACACTACCT
TCCAGGGTTT TCCAGCCCAC TTTCATCACC AAGGCCTCTC CACTTGTGAA
GAATTCCATC ACAAAGAACC AATGGCTCGT AACACCCAGC AGGGAATATG
CTACCAAGAC AAGAATTAGG ACTCACCGTG GGAAAACTGG ACAAGAACTG
AAAGAGGCAG CCAAGGAACC ATCAATGGAA AAAATCTTTA AAATCGATCA
AATGGGAAGG TGGTTTGTTG CTGGAGGAGC AGCTGTTGGT CTTGGAGCGC
TCTGCTACTA TGGCTTGGGA ATGTCTAATG AGATTGGAGC TATCGAAAAG
GCTGTAATTT GGCCTCAGTA TGTAAAGGAT AGAATTCATT CTACTTACAT
GTACTTAGCA GGAAGTATTG GTTTAACAGC TTTGTCTGCC TTGGCAGTAG
CCAGAACACC TGCTCTCATG AACTTCATGA TGACAGGCTC TTGGGTGACA
ATTGGTGCGA CCTTTGCAGC CATGATTGGA GCTGGAATGC TTGTACACTC
AATATCATAT GAGCAGAGCC CAGGCCCAAA GCATCTGGCT TGGATGCTGC
ATTCTGGTGT GATGGGTGCA GTTGTGGCTC CTCTGACGAT CTTAGGGGGG
CCTCTTCTCC TGAGAGCCGC ATGGTACACC GCTGGTATTG TGGGAGGCCT
CTCTACTGTG GCCATGTGTG CGCCTAGTGA GAAGTTTCTG AACATGGGAG
CACCCCTGGG AGTGGGCCTG GGTCTTGTCT TTGCGTCTTC TCTGGGGTCT
ATGTTTCTTC CCCCTACCTC TGTGGCTGGT GCCACTCTGT ACTCAGTGGC
AATGTATGGT GGATTAGTTC TTTTCAGCAT GTTCCTTCTG TATGATACTC
AGAAAGTAAT CAAACGTGCA GAAATAACAC CCATGTATGG AGCTCAAAAG
TATGATCCCA TCAATTCGAT GTTGACAATC TACATGGATA CATTAAATAT
ATTTATGCGA GTTGCAACTA TGCTAGCAAC TGGAAGCAAC AGAAAGAAAT
GAAGTAACCG CTTGTGATGT CTCCGCTCAC TGATGTCTTG CTTGTTTAAT
AGGAGCAGAT AGTCATTACA GTTTGCATCA GCAGAATTCC TTGAGGTTTA
GAAGATAGCC TGTCACCATG TTTAAAATGT GCAGTAATGC GACCCTTCAG
GCATGCCTTT TCTTTTAGAA AATAAATGCA ATAGATGTCT TCCAAATATA
TTTTCATCTC TTATGCTTTC ATACTTTAAA ACTGCTTTGA TGAATGTGTG
AACAAATATA TTTTAGAAGA TTTCAAGTAT TGTTTTATGT ATTGGATAAG
TAAAATTTAG CAAATTTGCG TGTCTTCATA TTGTGGAAGC CTGCAGAATA

TTTCAGTGGC ATCATGAGTG ACAAGTTTTT TGTATAGAGG TCAGAGAGAT
AAAAGGCACC TGCAGTCAGT TTGAATGCCC AGGACAACAC TGATTGTGGT
GAGCCAGTGA AAGACATCAG AGATGTGGAA CAAGGGACCA CCAAATGTGG
GGTTAACAAA GACACGGATG TTTCTTCTGT GCTCTTAATG TCCTTGAGGT
TGACTGCTCA TTGTCAGGAC AGTCCAGAGT GTTAACCATA CAGAGAATCT
CTGCTGGAAT TATGTCTGTG TTTTACTATG AAGTCTTTAG AACAAGCAGG
TTGGTGGTGG CGCACACCTT TAGTCCCATC ATCTGGGAGG CAGAGGCAAG
CAGATCTCTA AATTCAAGGC CAGCCAGGTC TACAAAGTGA GTTCCAAGCC
AGACAAGGAC CTGTCTCTAA TACAAGCAAA CAAACAACAA CAAACACTAC
CGCTATGCTC GGTATGATGT **ACTACTCCAA AGCTCAAGAC TCCTTTGCTG**
TCAGATGTGT GGTGTATATG CAGTTGGACA GGATTTAGGT TTTGGTTTTT
GGTTTTGTTT TATTTTGATA TTTTTCTCAG TGTCTAATTG AAAGCATGCT
TGCTTTCTCA TCACAGCTTT GACAGCTGTC AGAAAAGCCT CTTTGTGGCT
TATGCTAAGA TTAGGATTGG TTTTTCTTCT AAAACTGTTG GCTTCCTCCG
TTCCCTCTCA GCTTAAGCAT GAACAAAGCA AATTTAGTTG ACCTTGGGAA
GTATTTGAAT GAAAACTGGA ATGGGGAGGT GCTCAGCTTC CTTGTGACAT
AAGATTTTAA TACAGATCAC TTGTTTGTGG TGAGGGGTTC TTCATTGAAG
TCTGTATGTA TTTGCAAAAT AACTATTTTT GAGAAGTATT TATTACAGTA
ATCCATAAGT AATTCTTTTA ATCACTTTAA AGTACACTGA ATGCTAATTT
CTGAAATAAA AGTTTCAGCT AAGTG

Table 2B

Open Reading Frame Sequence (346-aa) of Clone 42:

Codes (Helix, *Strand*, no prediction)

MLAARLV*C*LRTLPSRVFQPT*F*ITKASPLVKNSITKNQWL*V*TPSREYATKT*RIRT*H
RGKTGQELKEAALEPSMEKI*F*KIDQMGRWFVAGGAAVGLGA*LCYY*GLGMSNEIGA
IEKA*V*IWPQYVKDRIHST*YMYLA*GS*IG*LTALSALAVARTPALMNFM*M*TGS*WVTI*G
ATFAAMIGAG*MLVHS*ISYEQSPGPKHLAWMLHSGVMG*AVV*AP*LTI*LGGPLLLRAA
WY*TAGIV*GGLS*TVAM*CAPSEKFLNMGAPLGVG*LGLVFA*SSLGSMFLPPTSVAGA*T*
*LYSVA*MYGGL*VLF*SM*FLL*YDTQKVIKRAEITPMYGAQKYDPINSM*LTIY*MDTLNI
FMRVATMLATGSNRKK
//

Table 3A.

mouse Brown Adipose Tissue Reverse (NT-GHA) 62 cDNA (EST); 735 BP. (in bold)

mouse Brown Adipose Tissue Clone 58 cDNA (full-length); 1379 BP.

Start Codon: 151 (underlined)

Stop Codon: 411 (underlined)

Open Reading Frame: 86-aa

GACTTCCGGC AGACGGTCGG AGCATTTACG GCCGTGGTGC CGCAAAGGCC
TGGAGTGAGG CGGTCTGAGC AAGCTGTCGT CTGGACCCCA GACCTGCTGG
TGGTGAAGTA TATCATGTAT AAAAGTGGAT CAATTCCATG TTAAGTGAAA
ATGGCCAATT CGTTACGAGG AGAAGTACTG ACTCTTTATA AAAATCTGCT
GTATCTTGGA CGGGACTATC CAAAAGGAGC AGACTATTTT AAAAGGCGTT
TGAAGAACGT TTTCCTTAAA AACAAGGATG TGGAGGACCC AGAGAAGATC
AAAGAACTTA TCGCACGAGG AGAATTTGTA ATGAAGGAGC TAGAGGCCTT
GTACTTCCTT AGGAAATACA GAGCTATGAA GCAACGTTAC TATTCAGATA
CCAAAGTCTG ACCAATCATT GCACCAGTCG AGCTGACAAC CAGTGCTGGC
TGTTTGCCTG TACCAACTAT TAAAAAATAA TTCAGTTTAA AAGGGTGAGA
TACATGGTTT TTAAAAAAAT GAGTTGCCCT ACTGTACTGA AATAGGTTTC
AACCTTATTG ATACTGAGAG CTTTGCCCAT AATCCTTTTA TTACTGAAAT
AGTAACTTTA GT**ACCTTTCA TGATAATATA ATTTTGAAAG AAAATACACT**
TAATTTTTAA ACATGTTATA GCCAATTTTC TTAAGTCTAT TTCTTCATTT
ACTGATGAGA TTGTCACTAT CGAATGGTGT CTGACAGGCT TGCCCTTTAG
CTTCTAGAGT GTCTTTGTCC TTGTTTTTTG TTGTTTTGTT AGCCCATCTA
GTATACTAAA GTGCATATTC AAGGCTCTCT ACAGACACCT CAAAATGATT
TAAATGCAGT TATCAAAATA AGACATGTGA AGGTGACCTC TATCTTGAGA
AGCTCAGTGG GTGACTAGCA TTGTGTAGCT ATTATTCCCA TTATTCTTTG
TGCTGCTGGC CTGCCTTAAG TTCTGAACCA CTTCAAGTAG CTTTCATGAG
GAGTTGTAAT GTTCCTCTAT TTCTGCCATT AAAGCTGGTA TATTTTCTGT
CGACCTGTAA CCGAGTCCAT GTGGCAGTGG ACCTAACCCA GGCAGGACTG
TAAGTTTAAG CAAAAATGTT TATGTAATGT TTTTAGCAAC GTTATAAATA
ACATTTCTAA CTTAAAAGCT GCAAATAGTG TTGCTTATAG GATTCTGTAT
CAGGCTGGAG AGATGGCTCA GTGGTTAAGA GCACTGACTG CTCTTCCAGA
GGTCCTGAAT TTAATTCCCA GCAACCATAT GGTGGCTTAC AACCATCTGT
AATGGGATCT GATGTCCACT TCTGGTGTGT CTGAACACAG ACAGTGTACT
CATAGAATAA ATAAATAAAC GAATAAATC //

Table 3B

mouse Brown Adipose Tissue Reverse (NT-GHA) 62 cDNA (EST); 735 BP. (in bold)

mouse Brown Adipose Tissue Clone 65 cDNA full-length); 2436 BP.

Start Codon: 804 (underlined)
Stop Codon: 1064 (underlined)
Open Reading Frame: 86-aa

GAGACGGTCG GAGCATTTAC GGCCGTGGTG CCGCAAAGCG CTGGAGTGAG
GCGGTCTGAG CAAGCTGTCG TCTGGACCCC AGACCTGCTG GTGGTGAACT
AAAGCACCGA GTCAAAAGCA TGGTCAGCAG CATGGATGCT GTCTGCTCTG
CCTCCCGTGG AACCTTTCCA AGTGCTCCCT TTGCCCGCTG CCTCTTACTC
TGCATTCTCC TTAAGGACCA ACCTTCTTGA TCTTGATCGA ACAACCCAAT
TTATCTTAGT TTTAAAATTT CCTCCAAGAA TACTCTTCTA GATTTGGGCT
CTTAGTTTCT TCCAAATAAT CAAGCCAAGC CTTGAGAGCA GGGCAGACAG
CTTTACTTTT GGTAAGGAAA GCAGGCTTAG AAAAGTGGTG TTACCCAGTG
CCTCAATAAA ACAGCTCAGT ACAAATAACC ATTTGGGGGG ATAAGAAGTC
TTAATGGCAA AGCACTTGCA CAAACAAGAG GGTCCTGTAG ACCTGCAAGT
TTGTAATCCC AGTGTACATA CAGGGGGGTG AGAGGTAGGA GAATCCCTAA
ATGAAGGAAG GCCAGCTGT TTGCAGCAAC AACTAAGACC CGTGGAAAGG
ACTGACAGCT GAGGTCATCA GCTCCAAATG CACACTGGCA AGTACAAGTC
TGTACACAAG AATGAAAAGC CAGCTCACCA GCTCCATGGG AAGATCTCTG
GTTCTTTAAG ATTTACAATG CAGTTATTTG CAAAAAAAAG AAAATCTTCC
TTTTCTTTAG GTATATCATG TATAAAAGTG GATCAATTCC ATGTTAAGTG
AAAATGGCCA ATTCGTTACG AGGAGAAGTA CTGACTCTTT ATAAAAATCT
GCTGTATCTT GGACGGGACT ATCCAAAAGG AGCAGACTAT TTTAAAAGGC
GTTTGAAGAA CGTTTTCCTT AAAAACAAGG ATGTGGAGGA CCCAGAGAAG
ATCAAAGAAC TTATCGCACG AGGAGAATTT GTAATGAAGG AGCTAGAGGC
CTTGTACTTC CTTAGGAAAT ACAGAGCTAT GAAGCAACGT TACTATTCAG
ATACCAAAGT CTGACCAATC ATTGCACCAG TCGAGCTGAC AACCAGTGCT
GGCTGTTTGC CTGTACCAAC TATTAAAAAA TAATTCAGTT TAAAAGGGTG
AGATACATGG TTTTTAAAAA AATGAGTTGC CCTACTGTAC TGAAATAGGT
TTCAACCTTA TTGATACTGA GAGCTTTGCC CATAATCCTT TTATTACTGA
AATAGTAACT TTAGT**ACCTT TCATGATAAT ATAATTTTGA AAGAAAATAC**
ACTTAATTTT TAAACATGTT ATAGCCAATT TTCTTAAGTC TATTTCTTCA
TTTACTGATG AGATTGTCAC TATCGAATGG TGTCTGACAG GCTTGCCCTT

TAGCTTCTAG AGTGTCTTTG TCCTTGTTTT TTGTTGTTTT GTTAGCCCAT
CTAGTATACT AAAGTGCATA TTCAAGGCTC TCTACAGACA CCTCAAAATG
ATTTAAATGC AGTTATCAAA ATAAGACATG TGAAGGTGAC CTCTATCTTG
AGAAGCTCAG TGGGTGACTA GCATTGTGTA GCTATTATTC CCATTATTCT
TTGTGCTGCT GGCCTGCCTT AAGTTCTGAA CCACTTCAAG TAGCTTTCAT
GAGGAGTTGT AATGTTCCTC TATTTCTGCC ATTAAAGCTG GTATATTTTC
TGTCGACCTG TAACCGAGTC CATGTGGCAG TGGACCTAAC CCAGGCAGGA
CTGTAAGTTT AAGCAAAAAT GTTTATGTAA TGTTTTTAGC AACGTTATAA
ATAACATTTC TAACTTAAAA GCTGCAAATA GTGTTGCTTA TAGGATTCTG
TATCAGGCTG GAGAGATGGC TCAGTGGTTA AGAGCACTGA CTGCTCTTCC
AGAGGTCCTG AATTTAATTC CCAGCAACCA TATGGTGGCT TACAACCATC
TGTAATGGGA TCTGATGTCC ACTTCTGGTG TGTCTGAACA CAGACAGTGT
ACTCATAGAA TAAATAAATA AACGAATAAA TCTTAAAGTC TTAAAGGAGT
CTTTATCAAC TACCAAGCAG ACATTTCCAC CAAGAAATAC CTATAGCCAG
GATGGGGATG AGGCTCAGTG TTAAGTACTT GCCTAAGGAA CACGTGAGGC
TCCAAAATTG AGCCTTAACC ACAATTAAAA CTACATAATT ACACACTTCA
TAGTCACCAT AACTATTTTT ATTACATTAC AATGATTAGG AGCAGTACGG
TTCATGACAA AAATATTACA AATTTCAGAT CACTTCACAG CACGTACTCC
TATAAACATT TAAAAGTTAA TTTTAATTAA GAGTGGTCAC TTTTAAATTT
AATGTTTGAT ATGACCAACA TTCCCTAGGT CAGCGCAACC AAAGGATGGA
AAACAACTGG ATCACACTGC ATATGTCCCA TAACAA//

Table 3C.

mouse Brown Adipose Tissue Reverse (NT-GHA) 62 cDNA (EST); 735 BP. (in bold)

mouse Brown Adipose Tissue Clone 66 cDNA (full-length); 1612 BP.

Start Codon: 385 (underlined)

Stop Codon: 645 (underlined)

Open Reading Frame: 86-aa

GGCCGTGGTG CCGCAAAGCG CTGGAGTGAG GCGGTCTGAG CAAGCTGTCG
TCTGGACCCC AGACCTGCTG GTGGTGAACT AAAGCACCGA GTCAAAAGCA
TGGTCAGCAG CATGGATGCT GTCTGCTCTG CCTCCCGTGG AACCTTTCCA
AGTGCTCCCT TTGCCCGCTG CCTCTTACTC TGCATTCTCC TTAAGGACCA
ACCTTCTTGA TCTTGATCGA ACAACCCAAT TTATCTTAGT TTTAAAATTT
CCTCCAAGAA TACTCTTCTA GATTTGGACT CTTAGTTTCT TCCAAATAAT
CAAGCCAAGC CTTGAGAGCA GGGCAGACAG CTTTACTTTT GGTATATCAT
GTATAAAAGT GGATCAATTC CATGTTAAGT GAAAATGGCC AATTCGTTAC
GAGGAGAAGT ACTGACTCTT TATAAAAATC TGCTGTATCT TGGACGGGAC
TATCCAAAAG GAGCAGACTA TTTTAAAAGG CGTTTGAAGA ACGTTTTCCT
TAAAAACAAG GATGTGGAGG ACCCAGAGAA GATCAAAGAA CTTATCGCAC
GAGGAGAATT TGTAATGAAG GAGCTAGAGG CCTTGTACTT CCTTAGGAAA
TACAGAGCTA TGAAGCAACG TTACTATTCA GATACCAAAG TCTGACCAAT
CATTGCACCA GTCGAGCTGA CAACCAGTGC TGGCTGTTTG CCTGTACCAA
CTATTAAAAA ATAATTCAGT TTAAAAGGGT GAGATACATG GTTTTTAAAA
AAATGAGTTG CCCTACTGTA CTGAAATAGG TTTCAACCTT ATTGATACTG
AGAGCTTTGC CCATAATCCT TTTATTACTG AAATAGTAAC TTTAGT**ACCT**
TTCATGATAA TATAATTTTG AAAGAAAATA CACTTAATTT TTAAACATGT
TATAGCCAAT TTTCTTAAGT CTATTTCTTC ATTTACTGAT GAGATTGTCA
CTATCGAATG GTGTCTGACA GGCTTGCCCT TTAGCTTCTA GAGTGTCTTT
GTCCTTGTTT TTTGTTGTTT TGTTAGCCCA TCTAGTATAC TAAAGTGCAT
ATTCAAGGCT CTCTACAGAC ACCTCAAAAT GATTTAAATG CAGTTATCAA
AATAAGACAT GTGAAGGTGA CCTCTATCTT GAGAAGCTCA GTGGGTGACT
AGCATTGTGT AGCTATTATT CCCATTATTC TTTGTGCTGC TGGCCTGCCT
TAAGTTCTGA ACCACTTCAA GTAGCTTTCA TGAGGAGTTG TAATGTTCCT
CTATTTCTGC CATTAAAGCT GGTATATTTT CTGTCGACCT GTAACCGAGT
CCATGTGGCA GTGGACCTAA CCCAGGCAGG ACTGTAAGTT TAAGCAAAAA
TGTTTATGTA ATGTTTTTAG CAACGTTATA AATAACATTT CTAACTTAAA

AGCTGCAAAT AGTGTTGCTT ATAGGATTCT GTATCAGGCT GGAGAGATGG
CTCAGTGGTT AAGAGCACTG ACTGCTCTTC CAGAGGTCCT GAATTTAATT
CCCAGCAACC ATATGGTGGC TTACAACCAT CTGTAATGGG ATCTGATGTC
CACTTCTGGT GTGTCTGAAC ACAGACAGTG TACTCATAGA ATAAATAAAT
AAACGAATAA AT//

Table 3D
Alignment of Clones 66, 58 and 65

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C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    GAGACGGTCG GAGCATTTAC GGCCGTGGTG CCGCAAAGCG CTGGAGTGAG      50

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    GCGGTCTGAG CAAGCTGTCG TCTGGACCCC AGACCTGCTG GTGGTGAACT     100

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    AAAGCACCGA GTCAAAAGCA TGGTCAGCAG CATGGATGCT GTCTGCTCTG     150

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    CCTCCCGTGG AACCTTTCCA AGTGCTCCCT TTGCCCGCTG CCTCTTACTC     200

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    TGCATTCTCC TTAAGGACCA ACCTTCTTGA TCTTGATCGA ACAACCCAAT     250

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    TTATCTTAGT TTTAAAATTT CCTCCAAGAA TACTCTTCTA GATTTGGGCT     300

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    CTTAGTTTCT TCCAAATAAT CAAGCCAAGC CTTGAGAGCA GGGCAGACAG     350

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    CTTTACTTTT GGTAAGGAAA GCAGGCTTAG AAAAGTGGTG TTACCCAGTG     400

C66    ---------- ---------- ---------- ---------- ----------
C58    ---------- ---------- ---------- ---------- ----------
C65    CCTCAATAAA ACAGCTCAGT ACAAATAACC ATTTGGGGGG ATAAGAAGTC     450

C66    -----GGCCG TGGTGCCGCA -AAGCGCTGG AGTGAGGCGG TCTGAGCAAG     44
C58    ---------- ---------- ---------- ---------- ----------
C65    TTAATGGCAA AGCACTTGCA CAAACAAGAG GGTCCTGTAG ACC-TGCAAG     499

C66    -CTGTCGT-C TGGACCCCAG ACCTGCTGGT G-G-TGAACT AAAGCACCGA     90
C58    ---------- ---------- ---------- ---------- ----------
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C65   TTTGTAATCC CAGTGTACAT ACAGGGGGGT GAGAGGTAGG AGAATCCCTA   549

C66   GTCAAAAGCA TGGTCAGCAG CATGGATGCT GTCTGCTCTG CCTCCCGTGG   140
C58   ---------- ---------- ---------- ---------- ----------
C65   AATGAAGGAA GGGCCAGCTG TTTGCA-GC- AACAACTAAG --ACCCGTGG   595

C66   AACCTTTCCA AGTGCTCCCT TTGCCCGCTG CCTCTTACTC TGCATTCTCC   190
C58   ---------- ---------- ---------- ---------- ----------
C65   AA-AGGACTG ACAGCTGAGG TCATCAGCT- CC----A-AA TGCACACT-G   637

C66   TTAAGGACCA ACCTTCTTGA TCTTGATCGA ACAACCCAAT TTATC-TTAG   239
C58   ---------- ---------- ---------- --------GA CTTCCGGCAG   12
C65   GCAAGTA-CA AGTCTGTACA -CAAGAATGA A-AAGCCAGC TCACCAGC--   682

C66   TT--TT--AA AATTTCCTCC AAGAATACTC TTCTAGATTT GGACTCTTAG   285
C58   ACGGTCGGAG CATTTACGGC -CGTGGTGCC GCAAAGGCCT GGAGT-GAGG   60
C65   TCCATGGGAA GATCT----- -C-TGGT-TC TTTAAGATTT ACAAT-GCAG   723

C66   TTTCTTCCAA ATAATCAAGC CAAGCCTTGA GAGCAGGGCA GA-CAGCTTT   334
C58   CG--GTC--- -TGAGCAAG- CTGTCGTCTG GACC---CCA GACCTGCTGG   100
C65   TT--ATT--- -T--GCAAA- AAAAAG--AA AATC---TT- --CCT-TT--   753

C66   ACTTTTGGTA TATCATGTAT AAAAGTGGAT CAATTCCATG TTAAGTGAAA   384
C58   TGGTGAAGTA TATCATGTAT AAAAGTGGAT CAATTCCATG TTAAGTGAAA   150
C65   TCTTTAGGTA TATCATGTAT AAAAGTGGAT CAATTCCATG TTAAGTGAAA   803

C66   ATGGCCAATT CGTTACGAGG AGAAGTACTG ACTCTTTATA AAAATCTGCT   434
C58   ATGGCCAATT CGTTACGAGG AGAAGTACTG ACTCTTTATA AAAATCTGCT   200
C65   ATGGCCAATT CGTTACGAGG AGAAGTACTG ACTCTTTATA AAAATCTGCT   853

C66   GTATCTTGGA CGGGACTATC CAAAAGGAGC AGACTATTTT AAAAGGCGTT   484
C58   GTATCTTGGA CGGGACTATC CAAAAGGAGC AGACTATTTT AAAAGGCGTT   250
C65   GTATCTTGGA CGGGACTATC CAAAAGGAGC AGACTATTTT AAAAGGCGTT   903

C66   TGAAGAACGT TTTCCTTAAA AACAAGGATG TGGAGGACCC AGAGAAGATC   534
C58   TGAAGAACGT TTTCCTTAAA AACAAGGATG TGGAGGACCC AGAGAAGATC   300
C65   TGAAGAACGT TTTCCTTAAA AACAAGGATG TGGAGGACCC AGAGAAGATC   953

C66   AAAGAACTTA TCGCACGAGG AGAATTTGTA ATGAAGGAGC TAGAGGCCTT   584
C58   AAAGAACTTA TCGCACGAGG AGAATTTGTA ATGAAGGAGC TAGAGGCCTT   350
C65   AAAGAACTTA TCGCACGAGG AGAATTTGTA ATGAAGGAGC TAGAGGCCTT   1003

C66   GTACTTCCTT AGGAAATACA GAGCTATGAA GCAACGTTAC TATTCAGATA   634
C58   GTACTTCCTT AGGAAATACA GAGCTATGAA GCAACGTTAC TATTCAGATA   400
C65   GTACTTCCTT AGGAAATACA GAGCTATGAA GCAACGTTAC TATTCAGATA   1053
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C66   CCAAAGTCTG ACCAATCATT GCACCAGTCG AGCTGACAAC CAGTGCTGGC   684
C58   CCAAAGTCTG ACCAATCATT GCACCAGTCG AGCTGACAAC CAGTGCTGGC   450
C65   CCAAAGTCTG ACCAATCATT GCACCAGTCG AGCTGACAAC CAGTGCTGGC   1103

C66   TGTTTGCCTG TACCAACTAT TAAAAAATAA TTCAGTTTAA AAGGGTGAGA   734
C58   TGTTTGCCTG TACCAACTAT TAAAAAATAA TTCAGTTTAA AAGGGTGAGA   500
C65   TGTTTGCCTG TACCAACTAT TAAAAAATAA TTCAGTTTAA AAGGGTGAGA   1153

C66   TACATGGTTT TTAAAAAAAT GAGTTGCCCT ACTGTACTGA AATAGGTTTC   784
C58   TACATGGTTT TTAAAAAAAT GAGTTGCCCT ACTGTACTGA AATAGGTTTC   550
C65   TACATGGTTT TTAAAAAAAT GAGTTGCCCT ACTGTACTGA AATAGGTTTC   1203

C66   AACCTTATTG ATACTGAGAG CTTTGCCCAT AATCCTTTTA TTACTGAAAT   834
C58   AACCTTATTG ATACTGAGAG CTTTGCCCAT AATCCTTTTA TTACTGAAAT   600
C65   AACCTTATTG ATACTGAGAG CTTTGCCCAT AATCCTTTTA TTACTGAAAT   1253

C66   AGTAACTTTA_GTACCTTTCA TGATAATATA ATTTTGAAAG AAAATACACT   884
C58   AGTAACTTTA_GTACCTTTCA TGATAATATA ATTTTGAAAG AAAATACACT   650
C65   AGTAACTTTA_GTACCTTTCA TGATAATATA ATTTTGAAAG AAAATACACT   1303

C66   TAATTTTTAA ACATGTTATA GCCAATTTTC TTAAGTCTAT TTCTTCATTT   934
C58   TAATTTTTAA ACATGTTATA GCCAATTTTC TTAAGTCTAT TTCTTCATTT   700
C65   TAATTTTTAA ACATGTTATA GCCAATTTTC TTAAGTCTAT TTCTTCATTT   1353

C66   ACTGATGAGA TTGTCACTAT CGAATGGTGT CTGACAGGCT TGCCCTTTAG   984
C58   ACTGATGAGA TTGTCACTAT CGAATGGTGT CTGACAGGCT TGCCCTTTAG   750
C65   ACTGATGAGA TTGTCACTAT CGAATGGTGT CTGACAGGCT TGCCCTTTAG   1403

C66   CTTCTAGAGT GTCTTTGTCC TTGTTTTTTG TTGTTTTGTT AGCCCATCTA   1034
C58   CTTCTAGAGT GTCTTTGTCC TTGTTTTTTG TTGTTTTGTT AGCCCATCTA   800
C65   CTTCTAGAGT GTCTTTGTCC TTGTTTTTTG TTGTTTTGTT AGCCCATCTA   1453

C66   GTATACTAAA GTGCATATTC AAGGCTCTCT ACAGACACCT CAAAATGATT   1084
C58   GTATACTAAA GTGCATATTC AAGGCTCTCT ACAGACACCT CAAAATGATT   850
C65   GTATACTAAA GTGCATATTC AAGGCTCTCT ACAGACACCT CAAAATGATT   1503

C66   TAAATGCAGT TATCAAAATA AGACATGTGA AGGTGACCTC TATCTTGAGA   1134
C58   TAAATGCAGT TATCAAAATA AGACATGTGA AGGTGACCTC TATCTTGAGA   900
C65   TAAATGCAGT TATCAAAATA AGACATGTGA AGGTGACCTC TATCTTGAGA   1553

C66   AGCTCAGTGG GTGACTAGCA TTGTGTAGCT ATTATTCCCA TTATTCTTTG   1184
C58   AGCTCAGTGG GTGACTAGCA TTGTGTAGCT ATTATTCCCA TTATTCTTTG   950
C65   AGCTCAGTGG GTGACTAGCA TTGTGTAGCT ATTATTCCCA TTATTCTTTG   1603
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C66   TGCTGCTGGC CTGCCTTAAG TTCTGAACCA CTTCAAGTAG CTTTCATGAG      1234
C58   TGCTGCTGGC CTGCCTTAAG TTCTGAACCA CTTCAAGTAG CTTTCATGAG      1000
C65   TGCTGCTGGC CTGCCTTAAG TTCTGAACCA CTTCAAGTAG CTTTCATGAG      1653

C66   GAGTTGTAAT GTTCCTCTAT TTCTGCCATT AAAGCTGGTA TATTTTCTGT      1284
C58   GAGTTGTAAT GTTCCTCTAT TTCTGCCATT AAAGCTGGTA TATTTTCTGT      1050
C65   GAGTTGTAAT GTTCCTCTAT TTCTGCCATT AAAGCTGGTA TATTTTCTGT      1703

C66   CGACCTGTAA CCGAGTCCAT GTGGCAGTGG ACCTAACCCA GGCAGGACTG      1334
C58   CGACCTGTAA CCGAGTCCAT GTGGCAGTGG ACCTAACCCA GGCAGGACTG      1100
C65   CGACCTGTAA CCGAGTCCAT GTGGCAGTGG ACCTAACCCA GGCAGGACTG      1753

C66   TAAGTTTAAG CAAAAATGTT TATGTAATGT TTTTAGCAAC GTTATAAATA      1384
C58   TAAGTTTAAG CAAAAATGTT TATGTAATGT TTTTAGCAAC GTTATAAATA      1150
C65   TAAGTTTAAG CAAAAATGTT TATGTAATGT TTTTAGCAAC GTTATAAATA      1803

C66   ACATTTCTAA CTTAAAAGCT GCAAATAGTG TTGCTTATAG GATTCTGTAT      1434
C58   ACATTTCTAA CTTAAAAGCT GCAAATAGTG TTGCTTATAG GATTCTGTAT      1200
C65   ACATTTCTAA CTTAAAAGCT GCAAATAGTG TTGCTTATAG GATTCTGTAT      1853

C66   CAGGCTGGAG AGATGGCTCA GTGGTTAAGA GCACTGACTG CTCTTCCAGA      1484
C58   CAGGCTGGAG AGATGGCTCA GTGGTTAAGA GCACTGACTG CTCTTCCAGA      1250
C65   CAGGCTGGAG AGATGGCTCA GTGGTTAAGA GCACTGACTG CTCTTCCAGA      1903

C66   GGTCCTGAAT TTAATTCCCA GCAACCATAT GGTGGCTTAC AACCATCTGT      1534
C58   GGTCCTGAAT TTAATTCCCA GCAACCATAT GGTGGCTTAC AACCATCTGT      1300
C65   GGTCCTGAAT TTAATTCCCA GCAACCATAT GGTGGCTTAC AACCATCTGT      1953

C66   AATGGGATCT GATGTCCACT TCTGGTGTGT CTGAACACAG ACAGTGTACT      1584
C58   AATGGGATCT GATGTCCACT TCTGGTGTGT CTGAACACAG ACAGTGTACT      1350
C65   AATGGGATCT GATGTCCACT TCTGGTGTGT CTGAACACAG ACAGTGTACT      2003

C66   CATAGAATAA ATAAATAAAC GAATAAAT-- ---------- ----------      1612
C58   CATAGAATAA ATAAATAAAC GAATAAATC- ---------- ----------      1379
C65   CATAGAATAA ATAAATAAAC GAATAAATCT TAAAGTCTTA AAGGAGTCTT      2053

C66   ---------- ---------- ---------- ---------- ----------      1612
C58   ---------- ---------- ---------- ---------- ----------      1379
C65   TATCAACTAC CAAGCAGACA TTTCCACCAA GAAATACCTA TAGCCAGGAT      2103

C66   ---------- ---------- ---------- ---------- ----------      1612
C58   ---------- ---------- ---------- ---------- ----------      1379
C65   GGGGATGAGG CTCAGTGTTA AGTACTTGCC TAAGGAACAC GTGAGGCTCC      2153

C66   ---------- ---------- ---------- ---------- ----------      1612
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C58   ---------- ---------- ---------- ---------- ----------   1379
C65   AAAATTGAGC CTTAACCACA ATTAAAACTA CATAATTACA CACTTCATAG   2203

C66   ---------- ---------- ---------- ---------- ----------   1612
C58   ---------- ---------- ---------- ---------- ----------   1379
C65   TCACCATAAC TATTTTTATT ACATTACAAT GATTAGGAGC AGTACGGTTC   2253

C66   ---------- ---------- ---------- ---------- ----------   1612
C58   ---------- ---------- ---------- ---------- ----------   1379
C65   ATGACAAAAA TATTACAAAT TTCAGATCAC TTCACAGCAC GTACTCCTAT   2303

C66   ---------- ---------- ---------- ---------- ----------   1612
C58   ---------- ---------- ---------- ---------- ----------   1379
C65   AAACATTTAA AAGTTAATTT TAATTAAGAG TGGTCACTTT TAAATTTAAT   2353

C66   ---------- ---------- ---------- ---------- ----------   1612
C58   ---------- ---------- ---------- ---------- ----------   1379
C65   GTTTGATATG ACCAACATTC CCTAGGTCAG CGCAACCAAA GGATGGAAAA   2403

C66   ---------- ---------- ---------- ---        1612
C58   ---------- ---------- ---------- ---        1379
C65   CAACTGGATC ACACTGCATA TGTCCCATAA CAA        2436
```

Position of probe 62 shown by underlining.

Table 3E

Open Reading Frame Sequence (86-aa) for clones 66, 58, 65:

MANSLRGEVLTLYKNLLYLGRDYPKGADYFKRRLKNVFLKNKDVEDPEKIKELIARGEFV
MKELEALYFLRKYRAMKQRYYSDTKV

Table 3F

Polypeptide Sequence Alignments:

Sequence 1: ORF of Clone 58, 65, & 66

Sequence 2: CG6115 gene encoding a 85-aa polypeptide in *Drosophila melanogaster* (GI: 7298358)

intermediate marking indicates the identities (letter) and similarities (+)

MANSLRGEVLTLYKNLLYLGRDYP--KGAD
M + LR +V++LYK+L YLGR+YPG
M-SQLRSKVISLYKHLQYLGREYPGLNGPQ

YFKRRLKNVFLKNKDVEDPEKIKELIARGE
F++++ + F+ +KD +DP+KI L+A+G
KFRKQIHDAFMNHKDEQDPKKIVALLAQGR

FVMKELEALYFLRKYRAMKQRY-YSDTKV
++ KE+EALY L+KYR++KQRY Y+D
YLAKEVEALYSLKKYRSVKQRYSYND

Table 4. Unknown Estimated Sequence Tags (EST) Identified in Reverse Subtraction Library (NT-GHA)

Table 4a.

mouse Brown Adipose Tissue Reverse (NT-GHA) 2 cDNA (EST); 556 BP.

1st strand:

ACATTTCAAG AGATGGAGAA ACATTTAGGT CCAGTAAATT TCTTGGTAAA
TGCAGCCGGT ATCAACAGAG ACAGTCTTCT AGTAAGAACA AAGACTGAAG
ACATGATCTC TCAGCTGCAC ACTAACCTCC TGGGCTCCAT GCTGACCTGT
AAAGCTGCCA TGGAGACAAT GATTCAGCAG GGAGGGTCTA TTGTTAATGT
GGGAAGTATT ATTGGTTTGA AAGGCAACGT TGGCCAGTCT GCATACAGTG
CCACCAAAGG AGGACTCGTT GGGTTTTCAC GCTCGCTTGC TAAAGAGGTT
GCACGGAAGA AAAATCAGAG TGAATGTGGT GGCACCAGGA TTTATTCGCA
CGGATATGAC AAGACACTTG AAAGAAGAAC ACTTCAAGAA AAACATTCCT
CTTGGGAGGT TTGGAGAAAC TCCTTGAGGT AGCACATGCC GTTGTGTTTC
TTTTAGAGTC ACCATACATC ACAGGCCATG TTCTTACCGT GGATGGAGGA
TTGCAGCTCA CCGTCTAATT AGAGATGATG TTACTGTGAT GCGCTTTGGG
TCAAGT
//

2nd strand:

ACTTGACCCA AAGCGCATCA CAGTAACATC ATCTCTAATT AGACGGTGAG
CTGCAATCCT CCATCCACGG TAAGAACATG GCCTGTGATG TATGGTGACT
CTAAAAGAAA CACAACGGCA TGTGCTACCT CAAGGAGTTT CTCCAAACCT
CCCAAGAGGA ATGTTTTTCT TGAAGTGTTC TTCTTTCAAG TGTCTTGTCA
TATCCGTGCG AATAAATCCT GGTGCCACCA CATTCACTCT GATTTTTCTT
CCGTGCAACC TCTTTAGCAA GCGAGCGTGA AAACCCAACG AGTCCTCCTT
TGGTGGCACT GTATGCAGAC TGGCCAACGT TGCCTTTCAA ACCAATAATA
CTTCCCACAT TAACAATAGA CCCTCCCTGC TGAATCATTG TCTCCATGGC
AGCTTTACAG GTCAGCATGG AGCCCAGGAG GTTAGTGTGC AGCTGAGAGA
TCATGTCTTC AGTCTTTGTT CTTACTAGAA GACTGTCTCT GTTGATACCG

GCTGCATTTA CCAAGAAATT TACTGGACCT AAATGTTTCT CCATCTCTTG AAATGT

Table 4B. mouse Brown Adipose Tissue Reverse (NT-GHA) 15 cDNA (EST); 681 BP.

1st strand:

ACCCATTAGC CAAACAGAAC TCCTGAATAT ATCTTGAAAG CCTTTCTTGT
ATTGTTTCTT CATCTGTAGG TTTGAACACA GCAGGAGATT TTATCATGGC
CTCCACCTGA TCCACCTCTA TTTCCCAGTC CCTAGCTAAT CTCTGCAAAG
ATGTTTCATC CACTCCAAAC ACAGTGCGGT AGAATTTCAT GCTTTTCTTC
AGAGTCTCCA AATCACTGTC CAAGAGAAAG GTCAGAGAAG GGATGATATT
CACTAGGTCA GCAGCAAATC CTTCCAGCCA AATCCTCTGC TTCAGAAATT
GCCGCTTCTT TTCAATGACT GAATCTGTGA TATTGGGTAA GGAGACCATA
AAATTGTGTC TCTTGTAGAT AGGGAGGTCA CTTATCAGCT TGTCCATCAG
GACGGGGAAG TCATAGTGAC AAACATTTTT GTTAGAGAGC AGGAAGATTG
GTGGCTCAGC AATGCCATTC TCCCTAAAGG TGTTCACACA GTTAAGGCGG
ATGTCCTGCA GGACCTTTTC TTTGTCAAAG GTTTGAGGTT TGCCATCTGC
TTCATTTGTT ATGTCAGAGT CCACCTTGGT TCTCACGAAG TAGAATTCCT
TCTTCATCAT GCTGATTGCT TTGGCAATGT CTATATCATT TTTCTTGAAG
CGTGTGGCCG AAATAATAAT GAAGAAATCG T //

2nd strand:

ACGATTTCTT CATTATTATT TCGGCCACAC GCTTCAAGAA AAATGATATA
GACATTGCCA AAGCAATCAG CATGATGAAG AAGGAATTCT ACTTCGTGAG
AACCAAGGTG GACTCTGACA TAACAAATGA AGCAGATGGC AAACCTCAAA
CCTTTGACAA AGAAAAGGTC CTGCAGGACA TCCGCCTTAA CTGTGTGAAC
ACCTTTAGGG AGAATGGCAT TGCTGAGCCA CCAATCTTCC TGCTCTCTAA
CAAAAATGTT TGTCACTATG ACTTCCCCGT CCTGATGGAC AAGCTGATAA
GTGACCTCCC TATCTACAAG AGACACAATT TTATGGTCTC CTTACCCAAT
ATCACAGATT CAGTCATTGA AAAGAAGCGG CAATTTCTGA AGCAGAGGAT
TTGGCTGGAA GGATTTGCTG CTGACCTAGT GAATATCATC CCTTCTCTGA
CCTTTCTCTT GGACAGTGAT TTGGAGACTC TGAAGAAAAG CATGAAATTC
TACCGCACTG TGTTTGGAGT GGATGAAACA TCTTTGCAGA GATTAGCTAG
GGACTGGGAA ATAGAGGTGG ATCAGGTGGA GGCCATGATA AAATCTCCTG

CTGTGTTCAA ACCTACAGAT GAAGAAACAA TACAAGAAAG GCTTTCAAGA
TATATTCAGG AGTTCTGTTT GGCTAATGGG T//

Table 5. Unknown Full Length cDNA Sequence Identified in Brown Adipose Tissue Full Length cDNA Library.

mouse Brown Adipose Tissue NG62D'4-2-1-4 cDNA (Full-Length); 2280 BP.

Start Codon: 163

Stop Codon: 2241

Open Reading Frame: 692-aa

GACAGTGGGA GAGGCAAAAT GGCCGCGGGA GTGGCGGCGA GTGGATCGCT
TCCCACAGCG GGCATTATAA TTGATTAGGT TTCTGATATC AAGATATCTT
CCTAAGAAGT AAATTAACAA GCCTCACGTT TCTGTGCAAA CACTGAGGAG
CCAGTTGGCA CCATGAAGGT CTTCTGTGGC CGTGCCAATC CTACCACGGG
ATCCCTGGAG TGGCTGGAGG AGGATGAACA CTATGATTAC CACCAGGAGA
TTGCCAGGTC ATCCTATGCC GACATGCTAC ATGACAAAGA CAGAAATATA
AAATACTACC AGGGTATCCG GGCAGCTGTG AGCAGGGTGA AAGACAGAGG
ACAGAAGGCC TTGGTTCTTG ACATTGGCAC TGGCACAGGC CTCTTGTCAA
TGATGGCAGT TACTGCAGGG GCTGACTTCT GCTATGCTAT CGAGGTTTTT
AAGCCTATGG CTGAGGCTGC TGTGAAGATT GTGGAGAGGA ATGGCTTCAG
TGATAAGATT AAAGTCATTA ACAAGCACTC CACTGAGGTG ACAGTCGGAC
CAGATGGTGA CTTGCCGTGT CGTGCTAACA TTCTGATCAC GGAGCTGTTT
GACACAGAGC TGATTGGGGA GGGAGCGCTG CCCTCTTATG AGCATGCACA
CAAGCATCTT GTCCAGGAAG ACTGCGAGGC AGTGCCACAC AGGGCAACTG
TCTATGCCCA GCTGGTGGAG TCCCGAAGGA TGTGGTCCTG GAACAAGCTG
TTTCCCGTCC GTGTCCGGAC GAGTCTAGGC GAGCAGGTCA TCGTCCCCCC
CTCAGAATTG GAGAGGTGTC CTGGTGCGCC TTCAGTCTGT GACATTCAGC
TGAACCAGGT GTCGCCTGCT GACTTCACTG TCCTCAGTGA TGTGCTGCCA
ATGTTCAGCG TGGACTTCAG CAAGCAAGTC AGCAGCTCGG CAGCGTGCCA
TAGCAGGCAG TTTGTACCTT TGGCGTCTGG CCAAGCACAG GTGGTTCTGT
CCTGGTGGGA CATTGAAATG GACCCTGAGG GCAAGATCAA GTGCACCATG
GCACCCTTTT GGGCACAGAC AGATCCGCAG GAGCTTCAGT GGCGGGACCA
CTGGATGCAG TGTGTGTACT TCCTGCCGCA GGAGGAGCCT GTTGTGCAGG
GCTCACCCCG GTGCCTGGTA GCCCACCATG ATGACTACTG TGTGTGGTAC
AGCCTTCAGA GAACCAGCCC TGATGAGAAC GACAGCGCCT ACCAAGTGCG
ACCTGTGTGT GACTGTCAGG CTCACTTGCT CTGGAACCGG CCTCGGTTTG
GAGAAATCAA TGATCAGGAC AGAACTGATC ACTATGCCCA GGCCCTGAGG
ACTGTGCTGC TGCCAGGTAG CGTCTGCCTT TGTGTGAGTG ATGGCAGTCT
CCTCTCCATG CTGGCCCATC ACCTCGGAGC GGAGCAGGTG TTTACAGTTG
AGAGTTCAGT AGCTTCCTAT AGACTGATGA AAAGGATCTT CAAGGTTAAC

CACTTGGAAG ATAAAATCAG TGTCATCAAT AAACGGCCTG AGTTGCTGAC
AGCTGCAGAC CTGGAGGGCA AGAAGGTCTC CCTCCTCCTG GGTGAACCCT
TTTTCACCAC CAGCCTGCTG CCATGGCACA ACCTGTACTT CTGGTATGTC
CGTACCTCTG TGGACCAGCA CCTAGCACCT GGAGCTGTGG TGATGCCTCA
GGCTGCCTCA CTGCATGCCG TGATTGTGGA GTTCAGGGAC CTGTGGCGGA
TCCGGAGTCC TTGCGGTGAC TGCGAAGGTT TTGATGTGCA CATCATGGAT
GATATGATCA AGCACTCCCT GGATTTCCGA GAGAGCAGAG AGGCAGAGCC
ACACCCACTG TGGGAATACC CCTGCAGAAG CCTCTCCAAG CCTCAAGAGA
TCCTGACTTT TGATTTCCAG CAGCCCATCC CCCAACAGCC TATGCAATCC
AAGGGCACAA TGGAGCTGAC AAGACCCGGG AAGAGCCATG GGGCTGTCCT
GTGGATGGAG TATCAGCTCA CTCCAGACAG CACGATCAGC ACTGGCCTCA
TAAACCCTGC AGAAGACAAG GGGGACTGCT GCTGGAACCC CCACTGCAAG
CAAGCTGTGT ACTTCCTCAG CACCACGCTG GATCTCAGAG TGCCTCTGAA
TGGCCCTCGG TCAGTCAGCT ATGTTGTGGA GTTTCACCCC CTCACTGGAG
ACATCACCAT GGAGTTTAGG CTTGCAGACA CCTTGAGCTG ATCTCTTATT
GAGAAATAAA ATGGCCAGCA GGCTGCAGAC
//

ORF Sequence:

MKVFCGRANPTTGSLEWLEEDEHYDYHQEIARSSYADMLHDKDRNIKYYQGIRAAVSRVK
DRGQKALVLDIGTGTGLLSMMAVTAGADFCYAIEVFKPMAEAAVKIVERNGFSDKIKVIN
KHSTEVTVGPDGDLPCRANILITELFDTELIGEGALPSYEHAHKHLVQEDCEAVPHRATV
YAQLVESRRMWSWNKLFPVRVRTSLGEQVIVPPSELERCPGAPSVCDIQLNQVSPADFTV
LSDVLPMFSVDFSKQVSSSAACHSRQFVPLASGQAQVVLSWWDIEMDPEGKIKCTMAPFW
AQTDPQELQWRDHWMQCVYFLPQEEPVVQGSPRCLVAHHDDYCVWYSLQRTSPDENDSAY
QVRPVCDCQAHLLWNRPRFGEINDQDRTDHYAQALRTVLLPGSVCLCVSDGSLLSMLAHH
LGAEQVFTVESSVASYRLMKRIFKVNHLEDKISVINKRPELLTAADLEGKKVSLLLGEPF
FTTSLLPWHNLYFWYVRTSVDQHLAPGAVVMPQAASLHAVIVEFRDLWRIRSPCGDCEGF
DVHIMDDMIKHSLDFRESREAEPHPLWEYPCRSLSKPQEILTFDFQQPIPQQPMQSKGTM
ELTRPGKSHGAVLWMEYQLTPDSTISTGLINPAEDKGDCCWNPHCKQAVYFLSTTLDLRV
PLNGPRSVSYVVEFHPLTGDITMEFRLADTLS

CLAIMS

1. A method of diagnosing abnormal levels of growth hormone (GH) activity in brown adipose tissue, or of predicting a change in the condition of the brown adipose tissue in response to abnormal levels of GH activity therein, which comprises

(A) obtaining a sample of brown adipose tissue,

(B) assaying messenger RNA of said sample, or complementary DNA reverse transcribed from said messenger RNA, to determine the level of transcriptional activity of one or more of the following genes in said cell:

genes encoding:
glucosephosphate isomerase
neuroleukin
pyruvate kinase
heme oxygenase
ubiquitin/ribosomal fusion protein
α-enolase
proteasome θ chain

trans-Golgi network protein
medium chain acyl-CoA dehydrogenase
adipocyte lipid binding protein
mitochondrial cytochrome c oxidase
mitochondrial NADH-ubiquonone oxidoreductase or
mitochondrial cytochrome b,
or
genes comprising or hybridizing to
Ng-G119K2
Ng-G119K15
Ng-G119K36
Ng-G119K62
Ng-G119K42
Ng-G119K58
Ng-G119K65 or
Ng-G119K66

(C) correlating that level of activity with the level of GH activity in brown adipose tissue or the expected change in the condition of the brown adipose tissue as a result of such GH activity.

2. A method of diagnosing abnormal levels of growth hormone (GH) activity in the brown adipose tissue, or of predicting a change in the condition of the brown adipose tissue in response to abnormal levels of GH activity therein, which comprises

(A) obtaining a sample from said patient, where said sample is expected to contain protein produced by the brown adipose tissue,

(B) assaying the protein in said sample to determine the level of

expression of one or more of the following proteins:
glucosephosphate isomerase
neuroleukin
pyruvate kinase
heme oxygenase
ubiquitin/ribosomal fusion protein
α-enolase
proteasome θ chain

trans-Golgi network protein
medium chain acyl-CoA dehydrogenase
adipocyte lipid binding protein
mitochondrial cytochrome c oxidase
mitochondrial NADH-ubiquonone oxidoreductase or
mitochondrial cytochrome b,
or
proteins encoded by genes comprising or hybridizing to
Ng-G119K2
Ng-G119K15
Ng-G119K36
Ng-G119K62
Ng-G119K42
Ng-G119K58
Ng-G119K65 or
Ng-G119K66

(C) correlating that level of expression with the level of GH activity in the brown adipose tissue or the expected change in the condition of the brown adipose tissue as a result of such GH activity.

3. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of glucosephosphate isomerase.

4. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of neuroleukin.

5. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of pyruvate kinase.

6. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of heme oxygenase.

7. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of ubiquitin/ribosomal fusion protein.

8. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of α-enolase.

9. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of proteasome θ

chain.

10. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of trans-Golgi network protein.

11. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of medium chain acyl-CoA dehydrogenase.

12. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of adipocyte lipid binding protein.

13. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of mitochondrial cytochrome c oxidase.

14. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of mitochondrial NADH-ubiquonone oxidoreductase.

15. The method of claim 1 or 2 which comprises determining the level of transcriptional activity or level of expression of mitochondrial cytochrome b.

FIG. 1A

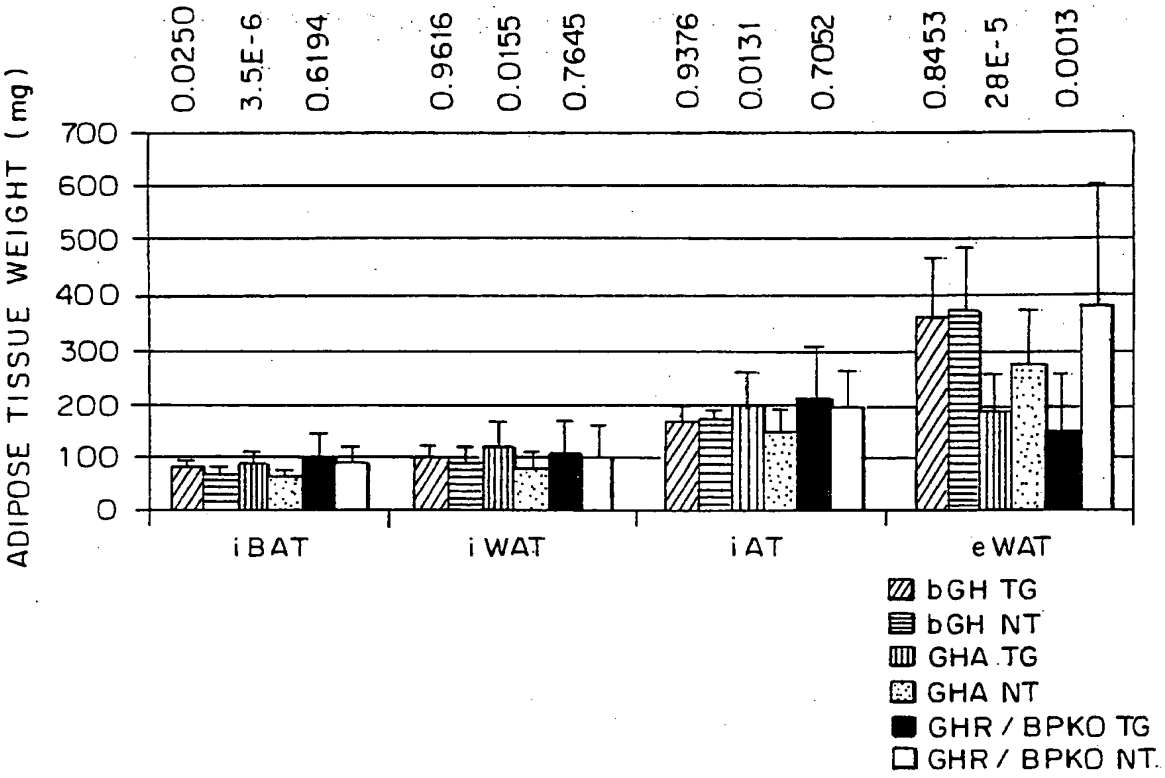
ADIPOSE TISSUE WEIGHT (mg)
700
600
500
400
300
200
100
0
0.0250
3.5E-6
0.6194
0.9616
0.0155
0.7645
0.9376
0.0131
0.7052
0.8453
28E-5
0.0013
iBAT
iWAT
iAT
eWAT
bGH TG
bGH NT
GHA TG
GHA NT
GHR / BPKO TG
GHR / BPKO NT

FIG. 1B

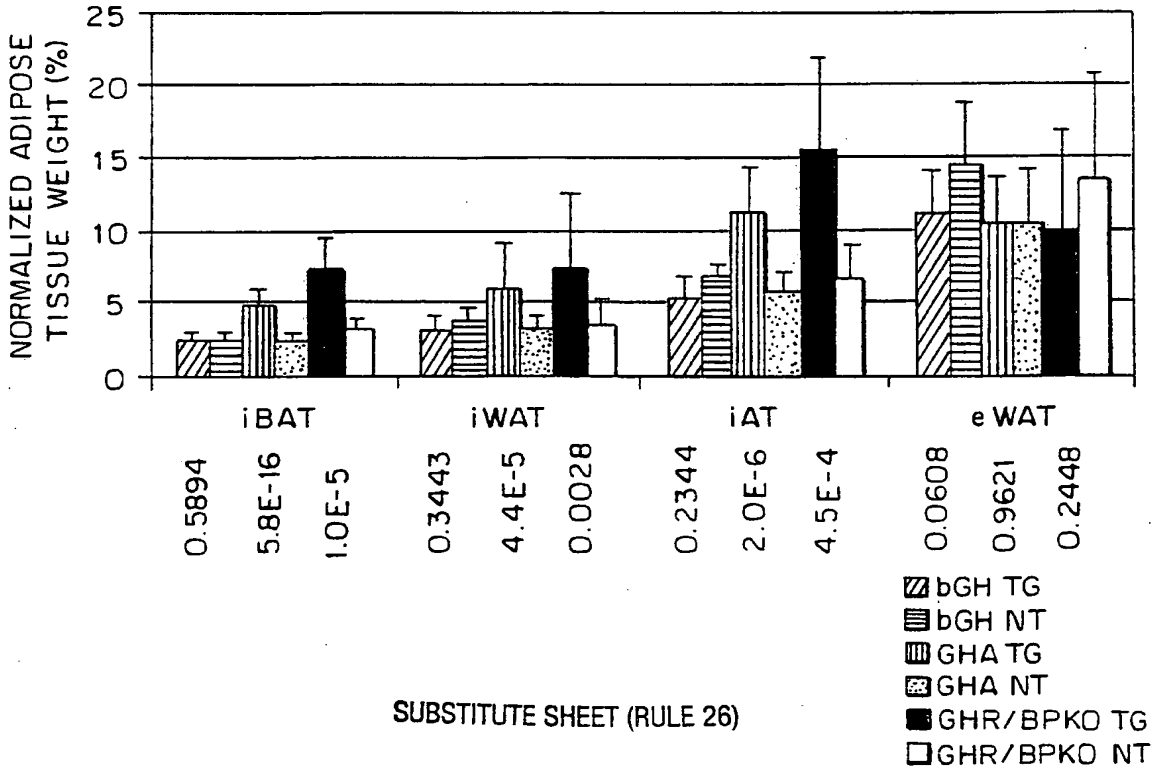
NORMALIZED ADIPOSE TISSUE WEIGHT (%)
25
20
15
10
5
0
iBAT
iWAT
iAT
eWAT
0.5894
5.8E-16
1.0E-5
0.3443
4.4E-5
0.0028
0.2344
2.0E-6
4.5E-4
0.0608
0.9621
0.2448
bGH TG
bGH NT
GHA TG
GHA NT
GHR/BPKO TG
GHR/BPKO NT

FIG. 2A

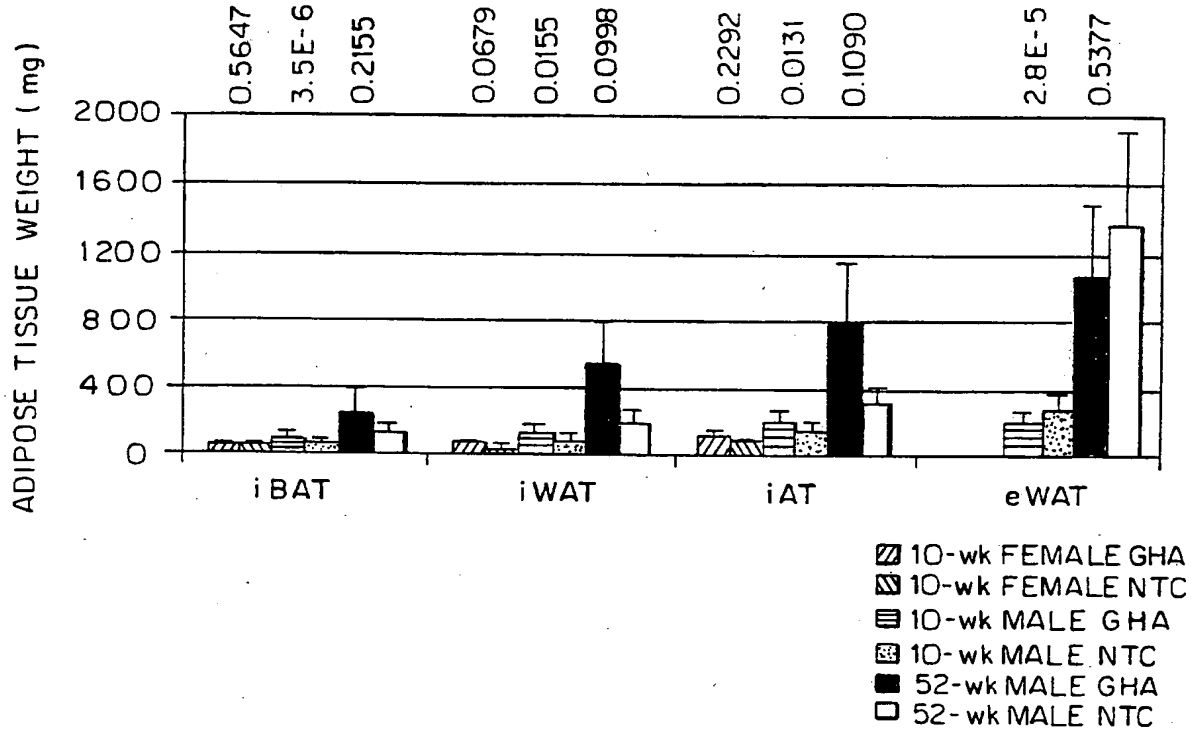
ADIPOSE TISSUE WEIGHT (mg)
2000
1600
1200
800
400
0
0.5647
3.5E-6
0.2155
0.0679
0.0155
0.0998
0.2292
0.0131
0.1090
2.8E-5
0.5377
iBAT
iWAT
iAT
eWAT
10-wk FEMALE GHA
10-wk FEMALE NTC
10-wk MALE GHA
10-wk MALE NTC
52-wk MALE GHA
52-wk MALE NTC

FIG. 2B

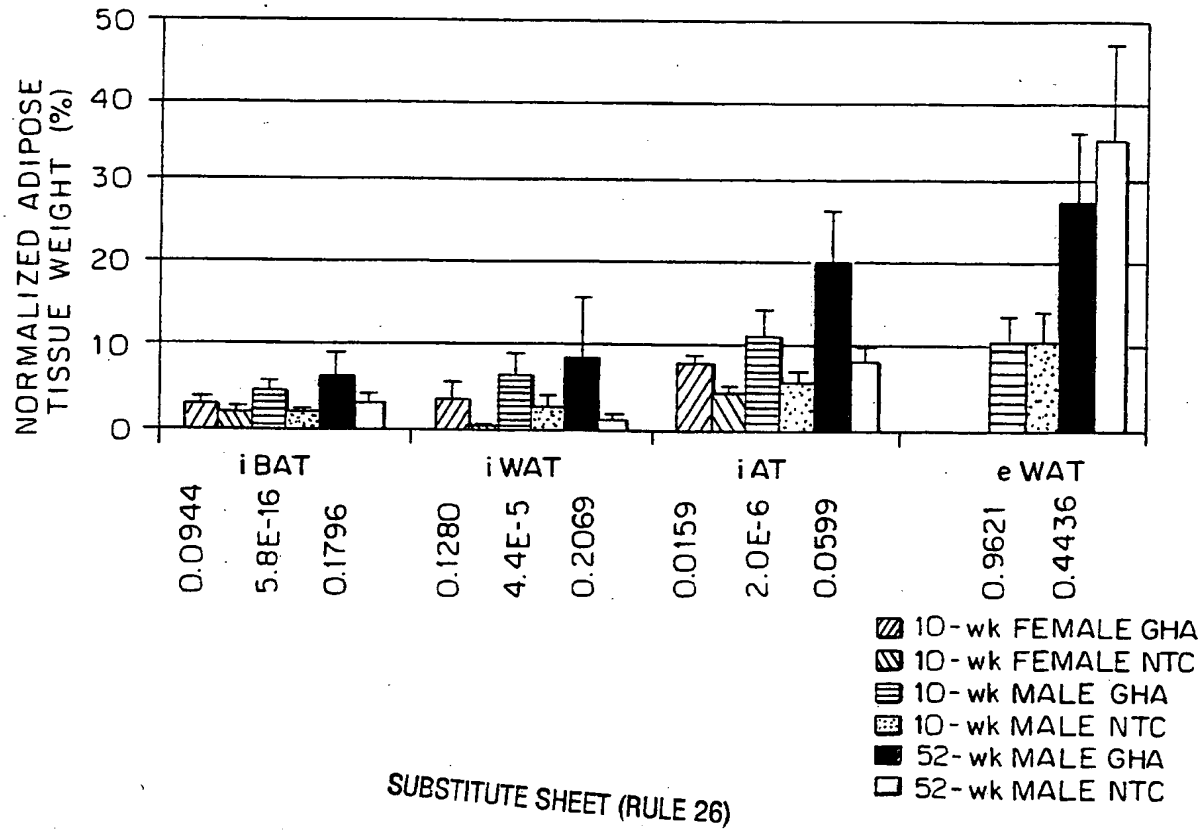
NORMALIZED ADIPOSE TISSUE WEIGHT (%)
50
40
30
20
10
0
iBAT
iWAT
iAT
eWAT
0.0944
5.8E-16
0.1796
0.1280
4.4E-5
0.2069
0.0159
2.0E-6
0.0599
0.9621
0.4436
10-wk FEMALE GHA
10-wk FEMALE NTC
10-wk MALE GHA
10-wk MALE NTC
52-wk MALE GHA
52-wk MALE NTC

Table C

Clone	Related Mouse Sequence	Identities
44	neuroleukin glucosephosphate isomerase	359/365 (98%) 265/275 (96%)
27	alpha-enolase	520/527 (98%) 359/363 (98%)
141	pyruvate kinase	291/303 (96%)
19 & 59	proteasome theta	255/268 (95%)
128	heme oxygenase	311/317 (98%)
30	ubiquitin	280/284 (98%)
68	trans-golgi network protein	48/49 (97%) 308/329 (93%)
99	adipocyte lipid binding protein	278/286 (97%) 211/224 (94%) 63/65 (96%)
123	same	355/364 (97%) 211/224 (94%) 103/105 (98%)
127	medium chain acyl-CoA dehydrogenase	540/542 (99%)
19	NADH-ubiquonone oxidoreductase	722/726 (99%)
160	same	130/131 (99%)
18	cytochrome b	596/598 (99%)
45	cytochrome c	323/334 (96%)